HUMAN ANTIBODY MOLECULES FOR IL-13

The present invention relates to specific binding members, in particular human anti-IL-13 antibody molecules and especially

5   those which neutralise IL-13 activity. It further relates to methods for using anti-IL-13 antibody molecules in diagnosis or treatment of IL-13 related disorders, including asthma, atopic dermatitis, allergic rhinitis, fibrosis, inflammatory bowel disease and Hodgkin's lymphoma.

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Preferred embodiments of the present invention employ the antibody VH and/or VL domain of the antibody molecule herein termed BAK502G9 and other antibody molecules of the BAK502G9 lineage and of the BAK278D6 lineage, as herein defined.

15   Further preferred embodiments employ complementarity determining regions (CDRs) of the BAK278D6 lineage, and preferably BAK502G9, especially VH CDR3 in other antibody framework regions. Further aspects of the present invention provide for compositions containing specific binding members

20   of the invention, and their use in methods of inhibiting or neutralising IL-13, including methods of treatment of the human or animal body by therapy.

The present invention provides antibody molecules of

25   particular value in binding and neutralising IL-13, and thus of use in any of a variety of therapeutic treatments, as indicated by the experimentation contained herein and further by the supporting technical literature.

30   Interleukin (IL)-13 is a 114 amino acid cytokine with an unmodified molecular mass of approximately 12 kDa [1,2]. IL-13 is most closely related to IL-4 with which it shares 30% sequence similarity at the amino acid level. The human IL-13 gene is located on chromosome 5q31 adjacent to the IL-4 gene

[1][2]. This region of chromosome 5q contains gene sequences
for other Th2 lymphocyte derived cytokines including GM-CSF
and IL-5, whose levels together with IL-4 have been shown to
correlate with disease severity in asthmatics and rodent

5   models of allergic inflammation [3][4][5][6][7][8].

Although initially identified as a Th2 CD4+ lymphocyte derived
cytokine, IL-13 is also produced by Th1 CD4+ T-cells, CD8+ T
lymphocytes NK cells, and non-T-cell populations such as mast

10   cells, basophils, eosinophils, macrophages, monocytes and
airway smooth muscle cells.

IL-13 is reported to mediate its effects through a receptor
system that includes the IL-4 receptor α chain (IL-4Rα), which

15   itself can bind IL-4 but not IL-13, and at least two other
cell surface proteins, IL-13Rα1 and IL-13Rα2 [9][10]. IL-13Rα1
can bind IL-13 with low affinity, subsequently recruiting IL-
4Rα to form a high affinity functional receptor that signals
[11][12]. The Genbank database lists the amino acid sequence

20   and the nucleic acid sequence of IL-13Rα1 as NP_001551 and
Y10659 respectively. Studies in STAT6 (signal transducer and
activator of transcription 6) -deficient mice have revealed
that IL-13, in a manner similar to IL-4, signals by utilising
the JAK-STAT6 pathway [13][14]. IL-13Rα2 shares 37% sequence

25   identity with IL-13Rα1 at the amino acid level and binds IL-13
with high affinity [15][16]. However, IL-13Rα2 has a shorter
cytoplasmic tail that lacks known signalling motifs. Cells
expressing IL-13Rα2 are not responsive to IL-13 even in the
presence of IL-4Rα [17]. It is postulated, therefore, that IL-

30   13Rα2 acts as a decoy receptor regulating IL-13 but not IL-4
function. This is supported by studies in IL-13Rα2 deficient
mice whose phenotype was consistent with increased
responsiveness to IL-13 [18][19]. The Genbank database lists

the amino acid sequence and the nucleic acid sequence of IL-13Rα2 as NP_000631 and Y08768 respectively.

5   The signalling IL-13Rα1/IL-4Rα receptor complex is expressed on human B-cells, mast cells, monocyte/macrophages, dendritic cells, eosinophils, basophils, fibroblasts, endothelial cells, airway epithelial cells and airway smooth muscle cells.

Bronchial asthma is a common persistent inflammatory disease
10   of the lung characterised by airways hyper-responsiveness, mucus overproduction, fibrosis and raised serum IgE levels. Airways hyper-responsiveness (AHR) is the exaggerated constriction of the airways to non-specific stimuli such as cold air. Both AHR and mucus overproduction are thought to be
15   responsible for the variable airway obstruction that leads to the shortness of breath characteristic of asthma attacks (exacerbations) and which is responsible for the mortality associated with this disease (around 2000 deaths/year in the United Kingdom).
20
The incidence of asthma, along with other allergic diseases, has increased significantly in recent years [20][21]. For example, currently, around 10% of the population of the United Kingdom (UK) has been diagnosed as asthmatic.
25
Current British Thoracic Society (BTS)and Global Initiative for Asthma (GINA) guidelines suggest a stepwise approach to the treatment of asthma [22, 23]. Mild to moderate asthma can usually be controlled by the use of inhaled corticosteroids,
30   in combination with beta-agonists or leukotriene inhibitors. However, due to the documented side effects of corticosteroids, patients tend not to comply with the treatment regime which reduces the effectiveness of treatment [24-26].

There is a clear need for new treatments for subjects with more severe disease, who often gain very limited benefit from either higher doses of inhaled or oral corticosteroids

5   recommended by asthma guidelines. Long term treatment with oral corticosteroids is associated with side effects such as osteoporosis, slowed growth rates in children, diabetes and oral candidiasis [88]. As both beneficial and adverse effects of corticosteroids are mediated via the same receptor,

10 treatment is a balance between safety and efficacy. Hospitalisation of these patients, who represent around 6% of the UK asthma population, as a result of severe exacerbations accounts for the majority of the significant economic burden of asthma on healthcare authorities [89].

15

It is believed that the pathology of asthma is caused by ongoing Th2 lymphocyte mediated inflammation that results from inappropriate responses of the immune system to harmless antigens. Evidence has been accrued which implicates IL-13,

20 rather than the classical Th2 derived cytokine IL-4, as the key mediator in the pathogenesis of established airway disease.

Administration of recombinant IL-13 to the airways of naïve

25 non-sensitised rodents caused many aspects of the asthma phenotype including airway inflammation, mucus production and AHR [27][28][29][30]. A similar phenotype was observed in a transgenic mouse in which IL-13 was specifically overexpressed in the lung. In this model more chronic exposure to IL-13 also

30 resulted in fibrosis [31].

Further, in rodent models of allergic disease many aspects of the asthma phenotype have been associated with IL-13. Soluble murine IL-13Rα2, a potent IL-13 neutraliser, has been shown to

inhibit AHR, mucus hypersecretion and the influx of
inflammatory cells which are characteristics of this rodent
model [27][28][30]. In complementary studies, mice in which
the IL-13 gene had been deleted, failed to develop allergen-

5   induced AHR. AHR could be restored in these IL-13 deficient
mice by the administration of recombinant IL-13. In contrast,
IL-4 deficient mice developed airway disease in this model
[32][33].

10   Using a longer-term allergen-induced pulmonary inflammation
model, Taube at al. demonstrated the efficacy of soluble
murine IL-13Rα2 against established airway disease [34].
Soluble murine IL-13Rα2 inhibited AHR, mucus overproduction
and to a lesser extent airway inflammation. In contrast,

15   soluble IL-4Rα, which binds and antagonises IL-4, had little
effect on AHR or airway inflammation in this system [35].
These findings were supported by Blease et al. who developed a
chronic fungal model of asthma in which polyclonal antibodies
against IL-13 but not IL-4 were able to reduce mucus

20   overproduction, AHR and subepithelial fibrosis [36].

A number of genetic polymorphisms in the IL-13 gene have also
been linked to allergic disease. In particular, a variant of
the IL-13 gene in which the arginine residue at amino acid 130

25   is substituted with glutamine (Q130R) has been associated with
bronchial asthma, atopic dermatitis and raised serum IgE
levels [37][38][39][40]. This particular IL-13 variant is also
referred to as the Q110R variant (arginine residue at amino
acid 110 is substituted with glutamine) by some groups who

30   exclude the 20 amino acid signal sequence from the amino acid
count. Arima et al, [41] report that this variant is
associated with raised levels of IL-13 in serum. The IL-13
variant (Q130R) and antibodies to this variant are discussed
in WO 01/62933. An IL-13 promoter polymorphism, which alters

IL-13 production, has also been associated with allergic asthma [42].

Raised levels of IL-13 have also been measured in human
5   subjects with asthma, atopic rhinitis (hay fever), allergic dermatitis (eczema) and chronic sinusitis. For example levels of IL-13 were found to be higher in bronchial biopsies, sputum and broncho-alveolar lavage (BAL) cells from asthmatics compared to control subjects [43][44][45][46]. Further, levels
10   of IL-13 in BAL samples increased in asthmatic individuals upon challenge with allergen [47][48]. The IL-13 production capacity of CD4(+) T cells has further been shown to be useful marker of risk for subsequent development of allergic disease in newborns [49].
15

Li et al [114] have recently reported affects of a neutralising anti-mouse IL-13 antibody in a chronic mouse model of asthma. Chronic asthma-like response (such as AHR, severe airway inflammation, hyper mucus productions) was
20   induced in OVA sensitised mice. Li et al report that administration of an IL-13 antibody at the time of each OVA challenge suppresses AHR, eosinophil infiltration, serum IgE levels, proinflammatory cytokine/chemokine levels and airway remodelling [14].
25

In summary, these data provide indication that IL-13 rather than IL-4 is a more attractive target for the treatment of human allergic disease.

30   IL-13 may play a role in the pathogenesis of inflammatory bowel disease. Heller et al.[116] report that neutralisation of IL-13 by administration of soluble IL-13Rα2 ameliorated colonic inflammation in a murine model of human ulcerative colitis [116]. Correspondingly, IL-13 expression was higher in

rectal biopsy specimens from ulcerative colitis patients when compared to controls [117].

Aside from asthma, IL-13 has been associated with other
5   fibrotic conditions. Increased levels of IL-13, up to a 1000
fold higher than IL-4, have been measured in the serum of
patients with systemic sclerosis [50] and in BAL samples from
patients affected with other forms of pulmonary fibrosis [51].
Correspondingly, overexpression of IL-13 but not IL-4 in the
10   mouse lung resulted in pronounced fibrosis [52][53]. The
contribution of IL-13 to fibrosis in tissues other than the
lung has been extensively studied in a mouse model of
parasite-induced liver fibrosis. Specific inhibition of IL-13
by administration of soluble IL-13Rα2 or IL-13 gene
15   disruption, but not ablation of IL-4 production prevented
fibrogenesis in the liver [54][55][56].

Chronic Obstructive Pulmonary Disease (COPD) includes patient
populations with varying degrees of chronic bronchitis, small
20   airway disease and emphysema and is characterised by
progressive irreversible lung function decline that responds
poorly to current asthma based therapy [90].
The incidence of COPD has risen dramatically in recent years
to become the fourth leading cause of death worldwide (World
25   Health Organisation). COPD therefore represents a large unmet
medical need.

The underlying causes of COPD remain poorly understood. The
"Dutch hypothesis" proposes that there is a common
30   susceptibility to COPD and asthma and therefore, that similar
mechanisms may contribute to the pathogenesis of both
disorders [57].

Zheng et al [58] have demonstrated that overexpression of IL-
13 in the mouse lung caused emphysema, elevated mucus
production and inflammation, reflecting aspects of human COPD.
Furthermore, AHR, an IL-13 dependent response in murine models

5   of allergic inflammation, has been shown to be predictive of
lung function decline in smokers [59]. A link has also been
established between an IL-13 promoter polymorphism and
susceptibility to develop COPD [60].

10   The signs are therefore that IL-13 plays an important role in
the pathogenesis of COPD, particularly in patients with
asthma-like features including AHR and eosinophilia. mRNA
levels of IL-13 have been shown to be higher in autopsy tissue
samples from subjects with a history of COPD when compared to

15   lung samples from subjects with no reported lung disease (J.
Elias, Oral communication at American Thoracic Society Annual
Meeting 2002). In another study, raised levels of IL-13 were
demonstrated by immunohistochemistry in peripheral lung
sections from COPD patients [91].

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Hodgkin's disease is a common type of lymphoma, which accounts
for approximately 7,500 cases per year in the United States.
Hodgkin's disease is unusual among malignancies in that the
neoplastic Reed-Sternberg cell, often derived from B-cells,

25   make up only a small proportion of the clinically detectable
mass. Hodgkin's disease-derived cell lines and primary Reed-
Sternberg cells frequently express IL-13 and its receptor
[61]. As IL-13 promotes cell survival and proliferation in
normal B-cells, it was proposed that IL-13 could act as a

30   growth factor for Reed-Sternberg cells. Skinnider et al. have
demonstrated that neutralising antibodies against IL-13 can
inhibit the growth of Hodgkin's disease-derived cell lines *in
vitro* [62]. This finding suggested that Reed-Sternberg cells
might enhance their own survival by an IL-13 autocrine and

paracrine cytokine loop. Consistent with this hypothesis, raised levels of IL-13 have been detected in the serum of some Hodgkin's disease patients when compared to normal controls [63]. IL-13 inhibitors may therefore prevent disease

5   progression by inhibiting proliferation of malignant Reed-Sternberg cells.

Many human cancer cells express immunogenic tumour specific antigens. However, although many tumours spontaneously

10 regress, a number evade the immune system (immunosurveillance) by suppressing T-cell mediated immunity. Terabe et al.[64] have demonstrated a role of IL-13 in immunosuppression in a mouse model in which tumours spontaneously regress after initial growth and then recur. Specific inhibition of IL-13,

15 with soluble IL-13Rα2, protected these mice from tumour recurrence. Terabe et al [64] went on to show that IL-13 suppresses the differentiation of tumour specific CD8+ cytotoxic lymphocytes that mediate anti-tumour immune responses.

20

IL-13 inhibitors may, therefore, be used therapeutically to prevent tumour recurrence or metastasis. Inhibition of IL-13 has been shown to enhance anti-viral vaccines in animal models and may be beneficial in the treatment of HIV and other

25 infectious diseases [65].

It should be noted that generally herein reference to interleukin-13 or IL-13 is, except where context dictates otherwise, reference to human IL-13. This is also referred to

30 in places as "the antigen". The present invention provides antibodies to human IL-13, especially human antibodies, that are cross-reactive with non-human primate IL-13, including cynomolgus and rhesus monkey IL-13. Antibodies in accordance with some embodiments of the present invention recognise a

variant of IL-13 in which the arginine residue at amino acid position 130 is replaced by glutamine. In other aspects and · embodiments the present invention provides specific binding members against murine IL-13, specifically mouse IL-13.

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Brief Description of Figures

Figure 1 shows neutralisation potency (% inhibition) of BAK167A11 (closed squares) and its derivative BAK615E3 (open

10   squares) as scFv against 25 ng/ml human IL-13 in the TF-1 cell proliferation assay. The triangles represent an irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

15   Figure 2 shows the neutralisation potency (% inhibition) of BAK278D6 (closed squares) and its derivative BAK502G9 (open squares) as scFv against 25 ng/ml human IL-13 in the TF-1 cell proliferation assay. The triangles represent an irrelevant scFv. Data represent the mean with standard error bars of

20   triplicate determinations within the same experiment.

Figure 3 shows the neutralisation potency (% inhibition) of BAK209B11 (closed squares) as a scFv against 25 ng/ml murine IL-13 in the TF-1 cell proliferation assay. The triangles

25   represent an irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 4 shows the neutralisation potency (% inhibition) of

30   BAK278D6 (closed squares) as a scFv against IL-13 in the TF-1 cell proliferation assay. The triangles represent an irrelevant scFv. Data represent the mean with standard error bars of triplicate determinations within the same experiment. Figure 4A show potency against 25 ng/ml human IL-13.

Figure 4B shows potency against 25 ng/ml human variant
IL-13.
Figure 4C shows potency against 50 ng/ml non-human
primate IL-13.

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Figure 5 shows a comparison of the potency of anti-human IL-13
antibodies in the TF-1 proliferation assay. Data represent the
mean neutralisation potency with standard error bars over 5-7
experiments against 25 ng/ml human IL-13. The performance
10   relative to the commercially available antibody, B-B13, was
evaluated statistically by performing a one-way ANOVA with
Dunnett's test. * $P<0.05$, ** $P<0.01$ compared to B-B13.

Figure 6 shows the neutralisation potency (% inhibition) of
15   BAK502G9 (closed squares), BAK1167F2 (closed triangles) and
BAK1183H4 (closed inverted triangles) as human IgG4 against
tagged IL-13 in the TF-1 cell proliferation assay. Open
triangles represent an irrelevant IgG4. Data represent the
mean with standard error bars of three separate experiments.
20   Figure 6A shows potency against 25 ng/ml human IL-13.
Figure 6B shows potency against 25 ng/ml human variant
IL-13.
Figure 6C shows potency against 50 ng/ml non-human
primate IL-13.

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Figure 7 shows the neutralisation potency (% inhibition) of
BAK502G9 (closed squares), BAK1167F2 (closed triangles),
BAK1183H4 (closed inverted triangles) as human IgG4 and
commercial anti-human IL-13 antibodies (B-B13 - open squares;
30   JES10-5A2 - open inverted triangles) in the native IL-13
dependent HDLM-2 cell proliferation assay. Open triangles
represent an irrelevant IgG4. Data represent the mean with
standard error bars of triplicate determinations within the
same experiment.

Figure 8 shows a comparison of the potency of anti-human IL-13 antibodies in the NHLF assay. Data represent the mean neutralisation potency (IC_{50} pM) with standard error bars over

5   4-5 experiments against 10 ng/ml human IL-13 in the NHLF eotaxin release assay. The performance relative to the commercially available antibody, B-B13, was evaluated statistically by performing a one-way ANOVA with Dunnett's test. * P<0.05, ** P<0.01 compared to B-B13.

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Figure 9 shows the neutralisation potency (% inhibition) of BAK502G9 (closed squares), BAK1167F2 (closed triangles), BAK1183H4 (closed inverted triangles) as human IgG4 against VCAM-1 upregulation on the surface of HUVEC in response to

15   10ng/ml human IL-13. Open triangles represent irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 10 shows the neutralisation potency (% inhibition) of

20   BAK502G9 (closed squares), BAK1167F2 (closed triangles), BAK1183H4 (closed inverted triangles) as human IgG4 against eotaxin release from VCAM-1 upregulation on the surface of HUVEC in response to either 1ng/ml human IL-4 (Figure 10A) or 0.5ng/ml human IL-1β (Figure 10B). Open triangles represent an

25   irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 11 shows the neutralisation potency (% inhibition) of BAK209B11 (squares) as a human IgG4 against 1 ng/ml murine IL-

30   13 in the factor dependent B9 cell proliferation assay. Open triangles represent an irrelevant IgG4. Data represent the mean with standard error bars of triplicate determinations within the same experiment.

Figure 12 shows the relative level of IL-13 in lung
homogenates from sensitised (s)(right-hand bar) and non-
sensitised (ns)(left-hand bar) mice post challenge in a murine
model of acute pulmonary allergic inflammation. The effect of
5   sensitisation was statistically evaluated by performing
Student's t-test using quantity of IL-13 data. *<0.05. **<0.01
compared to non-sensitised control animals (n=5-6 mice). Data
represent the mean with standard error bars.

10   Figure 13 illustrates the effects of i.v. administration of
BAK209B11 as human IgG4 in different amounts compared to an
isotype matched IgG4 irrelevant control antibody on ovalbumin
induced leukocyte recruitment to the lung in ovalbumin
sensitised mice. The number of leukocytes is shown (x 10^4).
15   The effect of antibody treatment was statistically evaluated
by performing one way ANOVA with Dunnett's test using
differential cell count data. *<0.05. **<0.01 compared to
ovalbumin challenged PBS control animals (=0% inhibition; n=5-
8 mice). Data represent the mean with standard error bars.
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Figure 14 illustrates the effects of i.v. administration of
BAK209B11 as human IgG4 in different amounts compared to an
isotype matched IgG4 irrelevant control antibody on ovalbumin
induced eosinophil recruitment to the lung in ovalbumin
25   sensitised mice. The number of eosinophils is shown (x 10^4).
The effect of antibody treatment was statistically evaluated
by performing one way ANOVA with Dunnett's test using
differential cell count data. *<0.05. **<0.01 compared to
ovalbumin challenged PBS control animals (=0% inhibition; n=5-
30   8 mice). Data represent the mean with standard error bars.

Figure 15 illustrates the effects of i.v. administration of
BAK209B11 as human IgG4 in different amounts compared to an
isotype matched IgG4 irrelevant control antibody on ovalbumin

induced neutrophil recruitment to the lung in ovalbumin
sensitised mice. The number of neutrophils is shown (x 10^4).
The effect of antibody treatment was statistically evaluated
by performing one way ANOVA with Dunnett's test using

5   differential cell count data. *<0.05. **<0.01 compared to
ovalbumin challenged PBS control animals (=0% inhibition; n=5-
8 mice). Data represent the mean with standard error bars.

Figure 16 illustrates the effects of i.v. administration of

10   BAK209B11 as human IgG4 in different amounts compared to an
isotype matched IgG4 irrelevant control antibody on ovalbumin
induced lymphocyte recruitment to the lung in ovalbumin
sensitised mice. The induction of lymphocytes was dose
dependently inhibited by BAK209B11 with maximal inhibition at

15   3µg/ml of BAK209B11. The effect of antibody treatment was
statistically evaluated by performing one way ANOVA with
Dunnett's test using differential cell count data. *<0.05.
**<0.01 compared to ovalbumin challenged PBS control animals
(=0% inhibition; n=5-8 mice). Data represent the mean with

20   standard error bars.

Figure 17 illustrates the effects of i.v. administration of
BAK209B11 as human IgG4 in different amounts compared to an
isotype matched IgG4 irrelevant control antibody on ovalbumin

25   induced monocyte/macrophage recruitment to the lung in
ovalbumin sensitised mice. There was no significant increase
in the levels of monocytes/macrophages of sensitised animals
when compared with control animals. However, such background
levels of these cells were depressed by ≥36µg/ml BAK209B11 in

30   sensitised animals. The effect of antibody treatment was
statistically evaluated by performing one way ANOVA with
Dunnett's test using differential cell count data. *<0.05.
**<0.01 compared to ovalbumin challenged PBS control animals

(=0% inhibition; n=5-8 mice). Data represent the mean with standard error bars.

5   Figure 18 shows the effects of a commercial anti-IL-13 neutralising antibody JES10-5A2 on the influx of cells (number of leukocytes is shown (x 10^4)) into the murine airpouch elicited by administration of bacterially derived recombinant human IL-13. The effect of antibody treatment was
10 statistically evaluated by performing one way ANOVA with Dunnett's test using differential cell count data. *<0.05. **<0.01 compared to CMC control animals (=0% inhibition; n=11-13 mice). Data represent the mean with standard error bars.

15 Figure 19 shows an sequence alignment of cynomolgus IL-13 against human IL-13 amino acid sequences. The seven amino acid residues that differ between human and cynomolgus IL-13 are shaded. Rhesus and cynomolgus IL-13 have an identical amino acid sequence.
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    Figure 20 illustrates the effects of single 10mg/kg i.v bolus dose of BAK502G9 as human IgG4 on serum IgE levels in 4 allergic but non-challenged cynomolgus primates (2 male/2 female) over 29 days. Serum IgE concentration is significantly
25 reduced from 100 % (predose) to 66 ± 10% of control values (p<0.05), at 4 and 5 days after dosing. This lowering of serum IgE concentration recovers to 88 ± 8 % of control levels by day 22. * = p<0.05 as compared to predose IgE levels, repeated measures ANOVA followed by Dunnett's multiple
30 comparisons test (n=4 animals).
    Figure 20B shows relative serum IgE levels of male and female cynomolgus primates versus time following a single 10mg/kg intravenous dose of BAK502G9. Relative serum IgE data are

expressed as arithmetic mean ± SEM percentage of baseline
value.

Figure 21 illustrates the effects of intraperitoneal
administration of BAK209B11 in different amounts ($H=237\mu g/day$,
$M=23.7\mu g/day$ and $L=2.37\mu g/day$) compared with an isotype
matched IgG1 irrelevant control antibody on the lung function
of ovalbumin sensitised and challenged mice. In Figure 21A
lung function is represented by log $PC_{50}s$ (log methacholine
concentration required to increase baseline PenH by 50%)
before any treatment (day 0) and post sensitisation, challenge
and drug treatment (day 25). Figure 21A shows the raw data
used to calculate the study endpoint, shown in figure 21B
(Delta log PC_{50}). Data represent the mean with standard error
bars of n=8.

In Figure 21B changing lung function is shown by a change in
an individual mouse's log PC_{50} (delta log PC_{50}). Delta log PC_{50}
is defined as an individuals change in log PC_{50} at day 25 verus
day 0. Data represent group mean delta log PC_{50} (individual
changes averaged within treatment groups) with standard error
bars. The effect of antibody treatment was statistically
evaluated by performing one way ANOVA with Dunnett's test
using delta log PC_{50} data. **$p<0.01$ compared to ovalbumin
sensitised and challenged control animals (n=8 mice).

Figure 22 illustrates the effects of local (i.po.) and
systemic (i.v.) administration of BAK502G9 as human IgG4 in
different amounts compared to an isotype matched IgG4
irrelevant control antibody on the total leukocyte recruitment
(Figure 22A) and eosinophil recruitment (Figure 22B) into the
air pouch of BALB/C mice. Data represent the mean with
standard error bars of n=10. The effect of antibody treatment
was statistically evaluated by performing one way ANOVA with

Dunnett's test using log-transformed data. *p<0.05, **p<0.01
compared to huIL-13 challenged mice (n=10).

Figure 23: illustrates the effects of i.p. administration of
5   BAK502G9 as human IgG4 compared to an isotype matched IgG4
irrelevant control antibody on the development of AHR
following intratracheal administration of human IL-13 to the
airways of mice. The effect of antibody treatment was
statistically evaluated by performing one way ANOVA with
10   Dunnett's test using PC_{200} Methacholine data. *<0.05. **<0.01
compared to the human IL-13 positive control group (n=6-8
mice). Data represent the mean with standard error bars.

Figure 24 shows the neutralisation potency (% maximal
15   response) of BAK502G9 (closed squares) as IgG4 against 30ng/ml
IL-13 in a human B cell IgE production assay. Open squares
represent an irrelevant IgG4. Data represent the mean with
standard error bars of six donors from separate experiments.

20   Figure 25 shows the effects of BAK502G9 on IL-13 induced
potentiation of agonist induced Ca^{2+} signalling in bronchial
smooth muscle cells. The area under the curve (AUC) of the Ca^{2+}
signalling response to histamine was determined for each
antibody +/- IL-13 pre-treatment condition. Combined data
25   from three independent experiments are shown for irrelevant
antibody CAT-001 (a) and BAK502G9 (b) as the percentage
difference versus untreated cells of AUC±SD (ns=not
significant (p>0.05), *p<0.05, **p<0.01). The results were
statistically evaluated utilising a one-way analysis of
30   variance (ANOVA) with Bonferroni's multiple comparisons post-
test.

Figure 26 shows effects of phase II administered BAK502G9.

Figure 26A shows effect on AHR as measured by change in area under the histamine dose response curve (n=14).

Figure 26B shows effect on AHR as measured by change in
5   PC_{30} (n=18).

Figure 26C shows effect on antigen priming (n=20).

Figure 26D shows effect on BAL inflammation (n=21).

Figure 27 shows effect of BAK502G9 on IL-13-induced CD23
10   expression. Data are presented as a percentage of the response to IL-13 alone (100%) and expressed as mean ± SEM % control of 6 separate experiments from 6 individual donors (performed in triplicate).

15   Figure 28 shows effect of BAK502G9 and irrelevant IgG4 on IL-13 and/or IL-4 induced PBMC CD23 expression. Data are presented as a percentage of the response to IL-4 alone (100%) and expressed as mean ± SEM % control of 4 separate experiments from 4 individual donors (performed in
20   triplicate).

Figure 29A shows effect of BAK502G9 on NHLF eotaxin-1 production induced by 48h culture with IL-13/ TNF-α/ TGF-β1 containing media. Data are shown as an arithmetic mean ± SEM
25   from triplicate determinations of the media used in this study to induce leukocyte shape change.

Figure 29B shows effect of BAK502G9 on shape change of human eosinophils induced by 1:16 diultion of conditioned media.
30   Data points represented are mean ± SEM %blank media shape change from separate experiments from four individual donors.

Figure 30 shows alignment of human IL-13 against murine IL13 highlighting the mutations that were introduced into human IL-

13 to produce the first panel of IL-13 chimaeras. The four
alpha helices are highlighted in boxes and loop 1 and loop 3
are labelled. Five chimeric proteins were produced where
helices B, C and D, and loops 1 and loop 3 were replaced with
5   the murine sequence. Four further chimeric proteins were
produced and numbered according to the amino acid in the human
pre-protein (not to the numbering of the multiple aligment
above) where arginine at residue 30 (position 34 above) was
mutated, residues 33 and 34 (position 37 and 38 above) were
10   mutated, residues 37 and 38 (VH) were mutated (position 41 and
42 above), 'and residues 40 and 41 (TQ) were mutated (position
44 and 45 above).

Figure 31 shows alignment of human IL-13 against murine IL-13
15   highlighting the mutations that were introduced into human IL-
13 to produce the second panel of IL-13 chimaeras. Six
chimaeras were produced where the human residue(s) were
substituted for the murine residue(s) (highlighted with
boxes). Four further chimeric proteins were produced
20   (numbering is according to the amino acid position in the
human pre-protein) where leucine at residue 58 (62 in above
figure) was mutated, leucine at. residue 119 (residue 123
above) was mutated, lysine at position 123 (residue 127 above)
was mutated, and arginine at residue 127 (residue 132 above
25   was mutated.

Figure 32 shows mutations made to human IL-13. Mutations in
dark grey reduced binding to BAK502G9, mutations in light grey
did not alter binding. Linear sequence of pre-human IL-13 with
30   the mutated residues indicated.

In various aspects and embodiments of the invention there is
provided the subject-matter of the claims included below.

The present invention provides specific binding members for
IL-13, in particular human and/or primate IL-13 and/or variant
IL-13 (Q130R), and murine IL-13. Preferred embodiments within
the present invention are antibody molecules, whether whole
5   antibody (e.g. IgG, such as IgG4) or antibody fragments (e.g.
scFv, Fab, dAb). Antibody antigen binding regions are
provided, as are antibody VH and VL domains. Within VH and VL
domains are provided complementarity determining regions,
CDR's, which may be provided within different framework
10   regions, FR's, to form VH or VL domains as the case may be.
An antigen binding site may consist of an antibody VH domain
and/or a VL domain.

An antigen binding site may be provided by means of
15   arrangement of CDR's on non-antibody protein scaffolds such as
fibronectin or cytochrome B etc. [115, 116]. Scaffolds for
engineering novel binding sites in proteins have been reviewed
in detail by Nygren et al [116]. Protein scaffolds for
antibody mimics are disclosed in WO/0034784 in which the
20   inventors describe proteins (antibody mimics) which include a
fibronectin type III domain having at least one randomised
loop. A suitable scaffold into which to graft one or more
CDR's, e.g. a set of HCDR's, may be provided by any domain
member of the immunoglobulin gene superfamily.
25

Preferred embodiments of the present invention are in what is
termed herein the "BAK278D6 lineage". This is defined with
reference to a set of six CDR sequences of BAK278D6 as
follows: HCDR1 (SEQ ID NO: 1), HCDR2 (SEQ ID NO: 2), HCDR3
30   (SEQ ID NO: 3), LCDR1 (SEQ ID NO: 4), LCDR2 (SEQ ID NO: 5) and
LCDR3 (SEQ ID NO: 6). In one aspect, the present invention
provides a specific binding member for human IL-13, comprising
an antibody antigen-binding site which is composed of a human
antibody VH domain and a human antibody VL domain and which

comprises a set of CDR's, wherein the VH domain comprises HCDR 1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID

5   NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6; or wherein the set of CDR's contains one or two amino acid substitutions

10   compared with the set of CDR's, wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid

15   sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6.

The set of CDR's wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 1, the HCDR2 has the amino acid sequence of SEQ

20   ID NO: 2, the HCDR3 has the amino acid sequence of SEQ ID NO: 3, the LCDR1 has the amino acid sequence of SEQ ID NO: 4, the LCDR2 has the amino acid sequence of SEQ ID NO: 5, and the LCDR3 has the amino acid sequence of SEQ ID NO: 6, are herein referred to as the "BAK278D6 set of CDR's". The HCDR1, HCDR2

25   and HCDR3 within the BAK278D6 set of CDR's are referred to as the "BAK278D6 set of HCDR's" and the LCDR1, LCDR2 and LCDR3 within the BAK278D6 set of CDR's are referred to as the "BAK278D6 set of LCDR's". A set of CDR's with the BAK278D6 set of CDR's, BAK278D6 set of HCDR's or BAK278D6 LCDR's, or

30   one or two substitutions therein, is said to be of the BAK278D6 lineage.

As noted, in one aspect the invention provides a specific binding member for human IL-13, comprising an antibody

antigen-binding site which is composed of a human antibody VH
domain and a human antibody VL domain and which comprises a
set of CDR's, wherein the set of CDR's is the BAK278D6 set of
CDR's or a set of CDR's containing one or two substitutions
5   compared with the BAK278D6 set of CDR's.

In preferred embodiments, the one or two substitutions are at
one or two of the following residues within the CDRs of the VH
and/or VL domains, using the standard numbering of Kabat
10   [107].

31, 32, 34 in HCDR1

52, 52A, 53, 54, 56, 58, 60, 61, 62, 64, 65 in HCDR2
15
96, 97, 98, 99, 101 in HCDR3

26, 27, 28, 30, 31 in LCDR1

20   56 in LCDR2

95A, 97 in LCDR3

Preferred embodiments have two substitutions compared with the
25   BAK278D6 set of CDR's, at HCDR3 residue 99 and LCDR1 residue
27.   Of these embodiments, preferred embodiments have S
substituted for N at HCDR3 residue 99 and/or I substituted for
N at LCDR 1 residue 27. Still further embodiments have a
substitution at HCDR3 residue 99 selected from the group
30   consisting of S, A, I, R, P and K, and/or a substitution at
LCDR1 residue 27 selected from the group consisting of I, L,
M, C, V, K, Y, F, R, T, S, A, H and G.

In preferred embodiments one or two substitutions are made at one or two of the following residues within the BAK278D6 set of CDR's in accordance with the identified groups of possible substitute residues:

5

Position of substitution	Substitute Residue selected from the group consisting of

10
31 in HCDR1:   Q, D, L, G and E

32 in HCDR1:   T

34 in HCDR1:   V, I and F

15
52 in HCDR2:   D, N, A, R, G and E

52A in HCDR2:   D, G, T, P, N and Y

53 in HCDR2:   D, L, A, P, T, S, I and R

54 in HCDR2:   S, T, D, G, K and I

56 in HCDR2:   T, E, Q, L, Y, N, V, A, M and G

20
58 in HCDR2:   I, L, Q, S, M, H, D and K

60 in HCDR2:   R

61 in HCDR2:   R

62 in HCDR2:   K and G

25
64 in HCDR2:   R

65 in HCDR2:   K

96 in HCDR3:   R and D

97 in HCDR3:   N, D, T and P

30
98 in HCDR3:   R

99 in HCDR3:   S, A, I, R, P and K

101 in HCDR3:   Y

26 in LCDR1:   D and S

27 in LCDR1:   I, L, M, C, V, K, Y, F, R, T, S, A, H and G

28 in LCDR1:   V

30 in LCDR1:   G

31 in LCDR1:   R

5

56 in LCDR2:   T

95A in LCDR3:   N

97 in LCDR3:   I

10

Preferred embodiments have the BAK278D6 set of CDR's with a
substitution of S for N at residue 99 within HCDR3 and I for N
at residue 27 within LCDR 1. The set of CDR's thus defined is
as follows: HCDR1 – SEQ ID NO: 7; HCDR2 – SEQ ID NO: 8, HCDR3

15   – SEQ ID NO: 9; LCDR1 – SEQ ID NO: 10, LCDR2 – SEQ ID NO: 11;
LCDR3 – SEQ ID NO: 12. This set of CDR's is herein referred
to as the "BAK502G9 set of CDR's".

Further preferred embodiments have the BAK278D6 set of CDR's

20   with one or two substitutions within the CDR's, with the
proviso that the pair of substitutions of S for N at residue
99 within HCDR3 and I for N at residue 27 within LCDR 1 is
excluded.

25   Other preferred embodiments are as follows: BAK 1166G2:
HCDR1– SEQ ID NO: 67, HCDR2– SEQ ID NO: 68, HCDR3– SEQ ID NO:
69, LCDR1 – SEQ ID NO: 70, LCDR2 – SEQ ID NO: 71; LCDR3 – SEQ
ID NO: 72.

30   BAK1167F2 HCDR1– SEQ ID NO: 61, HCDR2– SEQ ID NO:62, HCDR3–
SEQ ID NO:63, LCDR1 – SEQ ID NO: 64, LCDR2 – SEQ ID NO: 65;
LCDR3 – SEQ ID NO: 66.

BAK1184C8: HCDR1- SEQ ID NO:73, HCDR2: SEQ ID NO:74, HCDR3-
SEQ ID NO:75. LCDR1 - SEQ ID NO: 76, LCDR2 - SEQ ID NO: 77;
LCDR3 - SEQ ID NO: 78.

5   BAK1185E1: HCDR1- SEQ ID NO:79, HCDR2- SEQ ID NO:80, HCDR3-
SEQ ID NO: 81. LCDR1 - SEQ ID NO: 82, LCDR2 - SEQ ID NO: 83;
LCDR3 - SEQ ID NO: 84.

BAK1167F4: HCDR1- SEQ ID NO: 85, HCDR2- SEQ ID NO:86, HCDR3-
10   SEQ ID NO:87. LCDR1 - SEQ ID NO: 88, LCDR2 - SEQ ID NO: 89;
LCDR3 - SEQ ID NO: 90.

BAK1111D10: HCDR1- SEQ ID NO: 91, HCDR2- SEQ ID NO: 92, HCDR3-
SEQ ID NO: 93. LCDR1 - SEQ ID NO: 94, LCDR2 - SEQ ID NO: 95;
15   LCDR3 - SEQ ID NO: 96.

BAK1183H4: HCDR1- SEQ ID NO: 97, HCDR2- SEQ ID NO: 98, HCDR3-
SEQ ID NO: 99. LCDR1 - SEQ ID NO: 100, LCDR2 - SEQ ID NO: 101;
LCDR3 - SEQ ID NO: 102.
20

BAK1185F8: HCDR1- SEQ ID NO: 103, HCDR2- SEQ ID NO: 104,
HCDR3- SEQ ID NO: 105. LCDR1 - SEQ ID NO: 106, LCDR2 - SEQ ID
NO: 107; LCDR3 - SEQ ID NO: 108. All of these were derived
from BAK502G9 by heavy chain CDR1 and CDR2 randomisation and
25   are thus of the BAK502G9 lineage.

A VH domain comprising a set of CDR's HCDR1, HCDR2 and HCDR3
of any clone as shown in Table 1. Table 1 is also provided by
the present invention, as is separately a VL domain comprising
30   a set of CDR's LCDR1, LCDR2 and LCDR3 of the clones shown in
Table 1. Preferably such a VH domain is paired with such a VL
domain, and most preferably the VH and VL domain pairings are
the same as in the clones as set out in Table 1.

Further provided by the present invention is a VH domain comprising a set of CDR's HCDR1, HCDR2 and HCDR3 wherein the set of CDR's corresponds to that for any clone shown in Table 1 with one or two amino acid substitutions.

5

Further provided by the present invention is a VL domain comprising a set of CDR's LCDR1, LCDR2 and LCDR3 wherein the set of CDR's corresponds to that for any clone shown in Table 1 with one or two amino acid substitutions.

10

A specific binding member comprising an antibody antigen-binding domain comprising such a VH and/or VL domain is also provided by the present invention.

15 The present inventors have identified the BAK278D6 lineage as providing human antibody antigen-binding domains against IL-13 which are of particular value. Within the lineage, BAK502G9 has been identified to be of special value. The BAK278D6 and BAK502G9 sets of CDR's have been identified already above.

20

Following the lead of computational chemistry in applying multivariate data analysis techniques to the structure/property-activity relationships [94], quantitative activity-property relationships of antibodies can be derived

25 using well-known mathematical techniques such as statistical regression, pattern recognition and classification [95-100]. The properties of antibodies can be derived from empirical and theoretical models (for example, analysis of likely contact residues or calculated physicochemical property) of antibody

30 sequence, functional and three-dimensional structures and these properties can be considered singly and in combination.

An antibody antigen-binding site composed of a VH domain and a VL domain is formed by six loops of polypeptide: three from

the light chain variable domain (VL) and three from the heavy chain variable domain (VH). Analysis of antibodies of known atomic structure has elucidated relationships between the sequence and three-dimensional structure of antibody combining

5   sites[101,102]. These relationships imply that, except for the third region (loop) in VH domains, binding site loops have one of a small number of main-chain conformations: canonical structures. The canonical structure formed in a particular loop has been shown to be determined by its size and the

10   presence of certain residues at key sites in both the loop and in framework regions [101,102].

This study of sequence-structure relationship can be used for prediction of those residues in an antibody of known sequence,

15   but of an unknown three-dimensional structure, which are important in maintaining the three-dimensional structure of its CDR loops and hence maintain binding specificity. These predictions can be backed up by comparison of the predictions to the output from lead optimization experiments.

20

In a structural approach, a model can be created of the antibody molecule [103] using an freely available or commercial package such as WAM [104]. A protein visualisation and analysis software package such as Insight II [105] or Deep

25   View [106] may then be used to evaluate possible substitutions at each position in the CDR. This information may then be used to make substitutions likely to have a minimal or beneficial effect on activity.

30   The present inventors analysed sequence data of the panel of clones for which the sets of CDR's are shown in Table 1.

The analysis tested the hypothesis that any binary combinations of listed amino acid variations in the CDR's from

the presented set of scFv variants leads to a scFv variant
with at least the starting potency of the parent scFv
BAK278D6.

5   All scFv variants in the panel shown in Table 1 have been
selected for improved affinity and have been confirmed to
display higher potency.

The observed amino acid variations can either be favourable,
10   non-favourable or neutral in their effect on the starting
potency of scFv BAK278D6 in the TF-1 assay of 44nM.

No linkage was observed between any two amino acid variations
confirming that there was no synergy, either "positive" or
15   "negative", between any two selected amino acid variations.

There are four scenarios where such binary combination will
fulfil the hypothesis and three scenarios where the hypothesis
will not be valid. Synergistic amino acid variants are not
20   considered as no linkage was observed.

The hypothesis is valid where:

A1: mutation 1 is favourable and mutation 2 is favourable
25   A2 : mutation 1 is favourable and mutation 2 is neutral
A3: mutation 1 is neutral and mutation 2 is neutral
A4: mutation 1 is favourable and mutation 2 is non-favourable
(with the effect of 1 outweighing the effect of 2) .

30   The hypothesis is not valid where:

B1: mutation 1 is non-favourable and mutation 2 is neutral
B2: mutation 1 is non-favourable and mutation 2 is non-
favourable

B3: mutation 1 is favourable and mutation 2 is non-favourable
(with the effect of 2 outweighing the effect of 1).

For A4 to be possible, mutation 1 needs to be highly
favourable to counterbalance the negative effect of mutation 2
on potency. Since such highly favourable mutation would be
present in the library of variants used for selection, it
would be selected for and would therefore appear frequently in
the panel of variants. Since synergy can be excluded, such
mutation would be beneficial in any kind of sequence context
and should therefore reappear in different scFv variants. An
example for such frequent amino acid change is the change in
the light chain CDR1 Asn27Ile. However, this mutation on its
own (in clone BAK531E2) has only a modest 2-fold effect on
potency (final IC50 of 23.2nM). On its own this mutation would
not allow the scenario depicted in A4, as it is not a highly
favourable mutation. This suggests that every clone in the
presented set of IL-13 binding clones (Table 1) which has a
light chain CDR1 Asn27Ile change along with one or more
further mutations is at least as potent as the variant having
the single light chain CDR1 Asn27Ile mutation. The other
mutations are either neutral or positive but do not have a
negative or detrimental affect.

A further example is in the heavy chain CDR3 Asn99Ser (see
Table 1). As a clone carrying this particular single amino
acid variation is not observed, the potency of such a clone
has been estimated to be approximately 12.0nM by the following
rationale:

BAK278D6 potency is 44nM. Alterations of VL CDR1 N27I + VH
CDR3 N99S lead to BAK502G9 with potency 8nM, i.e. 5.5 fold
improvement.

BAK278D6 potency is 44nM. Alteration of VL CDR1 N27I leads to BAK531E2 with potency 23 nM, i.e. 1.9 fold improvement

BAK278D6 potency is 44nM. Alteration VH CDR3 N99S to provide
5   a possible clone with potency 12.2nM, i.e. 2.9 fold improvement (5.5/1.9 = 2.9).

The binary combination of heavy chain CDR3 Asn99Ser with light chain CDR1 Asn27Ile gives a scFv BAK0502G9 with a potency of
10 8nM. As synergy is excluded, the contribution of heavy chain CDR3 Asn99Ser change in BAK502G9 is therefore additive.

Therefore every clone in the presented set of IL-13 binding clones (Table 1) which has a heavy chain CDR3 AsnH99Ser change
15 along with one or more further mutations would have a potency of at least 12nM or greater, within a permissive assay window of 2.5-fold for n=1-2.

Thus, the inventors note that a highly favourable amino acid
20 variation which would be selected preferentially is not observed. As discussed above, two variations which were prominently represented in Table 1 of scFv variants were analysed closer. Any scFv variant in Table 1 with either of these mutations along with one or more further mutations
25 displayed a potency which was at least as improved as a clone containing any one of these two single amino acid variations in the parent BAK278D6. There is therefore no evidence that a highly favourable amino acid variation, that would allow scenario A4, is present in the panel.
30

This observation led the inventors to conclude that there were no non-favourable mutations present in this set of scFv variants. This means scenarios A4 and B1 to B3 are not relevant and the hypothesis is valid.

Accordingly, as noted already, the present invention provides
specific binding members comprising the defined sets of CDR's,
in particular the set of CDR's of BAK278D6, and sets of CDR's

5   of the BAK278D6 lineage, with one or two substitutions within
the set of CDR's, e.g. the BAK502G9 set of CDR's.

The relevant set of CDR's is provided within antibody
framework regions or other protein scaffold, e.g. fibronectin

10 or cytochrome B [115, 116]. Preferably antibody framework
regions are employed, and where they are employed they are
preferably germline, more preferably the antibody framework
region for the heavy chain may be DP14 from the VH1 family.
The preferred framework region for the light chain may be λ3-

15 3H.   For the BAK502G9 set of CDR's it is preferred that the
antibody framework regions are for VH FR1, SEQ ID NO: 27, for
VH FR2, SEQ ID NO: 28, for VH FR3, SEQ ID NO 29, for light
chain FR1, SEQ ID NO: 30, for light chain FR2, SEQ ID NO: 31,
for light chain FR3, SEQ ID NO: 32. In a highly preferred

20 embodiment, a VH domain is provided with the amino acid
sequence of SEQ ID NO: 15, this being termed "BAK502G9 VH
domain". In a further highly preferred embodiment, a VL
domain is provided with the amino acid sequence of SEQ ID NO:
16, this being termed "BAK502G9 VL domain". A highly

25 preferred antibody antigen-binding site provided in accordance
with the present invention is composed of the BAK502G9 VH
domain, SEQ ID NO: 15, and the BAK502G9 VL domain, SEQ ID NO:
16. This antibody antigen-binding site may be provided within
any desired antibody molecule format, e.g. scFv, Fab, IgG,

30 IgG4, dAb etc., as is discussed further elsewhere herein.

In a further highly preferred embodiment, the present
invention provides an IgG4 antibody molecule comprising the

BAK502G9 VH domain, SEQ ID NO: 15, and the BAK502G9 VL domain, SEQ ID NO: 16. This is termed herein "BAK502G9 IgG4".

5   Other IgG4 or other antibody molecules comprising the BAK502G9 VH domain, SEQ ID NO: 15, and/or the BAK502G9 VL domain, SEQ ID NO: 16, are provided by the present invention, as are other antibody molecules comprising the BAK502G9 set of HCDR's (SEQ ID NO: 7, 8 and 9) within an antibody VH domain, and/or the BAK502G9 set of LCDR's (SEQ ID NO: 10, 11 and 12) within an

10   antibody VL domain.

It is convenient to point out here that "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the

15   other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

As noted, the present invention provides a specific binding

20   member which binds human IL-13 and which comprises the BAK502G9 VH domain (SEQ ID NO: 15) and/or the BAK502G9 VL domain (SEQ ID NO: 16).

Generally, a VH domain is paired with a VL domain to provide

25   an antibody antigen binding site, although as discussed further below a VH domain alone may be used to bind antigen. In one preferred embodiment, the BAK502G9 VH domain (SEQ ID NO: 15) is paired with the BAK502G9 VL domain (SEQ ID NO: 16), so that an antibody antigen binding site is formed comprising

30   both the BAK502G9 VH and VL domains. In other embodiments, the BAK502G9 VH is paired with a VL domain other than the BAK502G9 VL. Light-chain promiscuity is well established in the art.

Similarly, any set of HCDR's of the BAK278D6 lineage can be
provided in a VH domain that is used as a specific binding
member alone or in combination with a VL domain.   A VH domain
may be provided with a set of HCDR's of a BAK278D6 lineage

5   antibody, e.g. as shown in Table 1, and if such a VH domain is
paired with a VL domain, then the VL domain may be provided
with a set of LCDR's of a BAK278D6 lineage antibody, e.g. as
shown in Table 1.   A pairing of a set of HCDR's and a set of
LCDR's may be as shown in Table 1, providing an antibody

10 antigen-binding site comprising a set of CDR's as shown in
Table 1.   The framework regions of the VH and/or VL domains
may be germline frameworks.   Frameworks regions of the heavy
chain domain may be selected from the VH-1 family, and a
preferred VH-1 framework is DP-14 framework. Framework regions

15 of the light chain may be selected from the λ3 family, and a
preferred such framework is λ3 3H.

One or more CDRs may be taken from the BAK502G9 VH or VL
domain and incorporated into a suitable framework.   This is

20 discussed further herein.   BAK502G9 HCDR's 1, 2 and 3 are
shown in SEQ ID NO: 7, 8 and 9, respectively.   BAK502G9 LCDR's
1, 2 and 3 are shown in SEQ ID NO: 10, 11 and 12,
respectively.

25 The same applies for other BAK278D6 lineage CDR's and sets of
CDR's as shown in Table 1.

Further embodiments of the invention relate to a specific
binding member comprising the VH and/or VL domain, or an

30 antigen binding site comprising CDRs of the VH and/or VL
domain of the antibody molecule disclosed herein as
167A11 (VH: SEQ ID NO: 23 and VL: SEQ ID NO: 24) and its
derivatives 615E3 (VH:SEQ ID NO: 33 and VL: SEQ ID NO: 34)
BAK582F7 (VH CDR's SEQ ID's 141-143) and BAK612B5 (VH CDR's

SEQ ID's 147-149). These recognise human IL-13. The
derivatives of 167A11 from VH CDR3 randomisation are potent
scFv molecules (5-6nM). The 167A11 lineage may be employed in
any aspect and embodiment of the present invention as
5   disclosed herein for other molecules, for instance methods of
mutation and selection of antigen binding sites with improved
potency.

Variants of the VH and VL domains and CDRs of the present
10 invention, including those for which amino acid sequences are
set out herein, and which can be employed in specific binding
members for IL-13 can be obtained by means of methods of
sequence alteration or mutation and screening. Such methods
are also provided by the present invention.
15

Variable domain amino acid sequence variants of any of the VH
and VL domains whose sequences are specifically disclosed
herein may be employed in accordance with the present
invention, as discussed. Particular variants may include one
20 or more amino acid sequence alterations (addition, deletion,
substitution and/or insertion of an amino acid residue), may
be less than about 20 alterations, less than about 15
alterations, less than about 10 alterations or less than about
5 alterations, 4, 3, 2 or 1. Alterations may be made in one
25 or more framework regions and/or one or more CDR's.

In accordance with further aspects of the present invention
there is provided a specific binding member which competes for
binding to antigen with any specific binding member which both
30 binds the antigen and comprises a specific binding member, VH
and/or VL domain disclosed herein, or HCDR3 disclosed herein,
or variant of any of these. Competition between binding
members may be assayed easily in vitro, for example using
ELISA and/or by tagging a specific reporter molecule to one

binding member which can be detected in the presence of other untagged binding member(s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope.

Thus, a further aspect of the present invention provides a specific binding member comprising a human antibody antigen-binding site which competes with a BAK502G9 antibody molecule, in particular BAK502G9 scFv and/or IgG4, for binding to IL-13. In further aspects the present invention provides a specific binding member comprising a human antibody antigen-binding site which competes with an antibody antigen-binding site for binding to IL-13, wherein the antibody antigen-binding site is composed of a VH domain and a VL domain, and wherein the VH and VL domains comprise a set of CDR's of the BAK278D6 lineage.

Various methods are available in the art for obtaining antibodies against IL-13 and which may compete with a BAK502G9 antibody molecule, an antibody molecule with a BAK502G9 set of CDR's, or an antibody molecule with a set of CDR's of BAK278D6 lineage, for binding to IL-13.

In a further aspect, the present invention provides a method of obtaining one or more specific binding members able to bind the antigen, the method including bringing into contact a library of specific binding members according to the invention and said antigen, and selecting one or more specific binding members of the library able to bind said antigen.

The library may be displayed on the surface of bacteriophage particles, each particle containing nucleic acid encoding the antibody VH variable domain displayed on its surface, and optionally also a displayed VL domain if present.

Following selection of specific binding members able to bind
the antigen and displayed on bacteriophage particles, nucleic
acid may be taken from a bacteriophage particle displaying a
said selected specific binding member. Such nucleic acid may
be used in subsequent production of a specific binding member
or an antibody VH variable domain (optionally an antibody VL
variable domain) by expression from nucleic acid with the
sequence of nucleic acid taken from a bacteriophage particle
displaying a said selected specific binding member.

An antibody VH variable domain with the amino acid sequence of
an antibody VH variable domain of a said selected specific
binding member may be provided in isolated form, as may a
specific binding member comprising such a VH domain.
Ability to bind IL-13 may be further tested, also ability to
compete with BAK502G9 (e.g. in scFv format and/or IgG format,
e.g. IgG4) for binding to IL-13. Ability to neutralise IL-13
may be tested, as discussed further below.

A specific binding member according to the present invention
may bind IL-13 with the affinity of a BAK502G9 antibody
molecule, e.g. scFv, or preferably BAK502G9 IgG4, or with an
affinity that is better.

A specific binding member according to the present invention
may neutralise IL-13 with the potency of a BAK502G9 antibody
molecule, e.g. scFv, or preferably BAK502G9 IgG4, or with a
potency that is better.

A specific binding member according to the present invention
may neutralise naturally occurring IL-13 with the potency of a
BAK502G9 antibody molecule, e.g. scFv, or preferably BAK502G9
IgG4, or with a potency that is better.

Binding affinity and neutralisation potency of different
specific binding members can be compared under appropriate
conditions.

5

The antibodies of the present invention have a number of
advantages over existing commercial anti-IL-13 antibodies, in
particular three commercial rodent anti-human IL-13 antibodies
namely, JES10-5A2 (BioSource), B-B13 (Euroclone) and clone

10   321166 (R&D Systems). The potency of the antibodies of the
present invention was compared with commercial antibodies
JES10-A2 and B-B13. Clone 321166 was not evaluated as previous
experiments revealed that this clone was considerably less
potent than other known commercial antibodies.

15

The efficacy and use of the rodent commercial IL-13 antibodies
in man is likely to be limited, because of their increased
potential to induce immunogenic responses and therefore more
rapid clearance from the body. Kinetic analysis of the

20   antibodies of the present invention in non-human primates
suggests that these antibodies have a clearance rate which is
similar to that of other known human or humanised antibodies.

Antibodies provided by various embodiments of the present

25   invention recognize non-human primate IL-13, including rhesus
and cynomolgus IL-13. Determining efficacy and safety profiles
of an antibody in non-human primates is extremely valuable as
it provides a means for predicting the antibody's safety,
pharmacokinetic and pharmacodynamic profile in humans.

30

Moreover, antibodies of various embodiments of the present
invention further recognize the human IL-13 variant, Q130R,
which is associated with asthma. Cross reactivity with variant
IL-13 allows antibodies of the present invention and

compositions comprising antibodies of the present invention to
be used for the treatment of patients with wild-type and
variant IL-13.

5   A preferred embodiment of the present invention comprises
     antibodies that neutralise naturally occurring IL-13 with a
     potency that is equal to or better than the potency of a IL-13
     antigen binding site formed by BAK502G9 VH domain (SEQ ID
     NO:15) and the BAK502G9 VL domain (SEQ ID NO: 16). For
10   example, the inventors have demonstrated that representative
     clones such as BAK502G9, 1167F2 and 1183H4 are significantly
     more potent against naturally occurring IL-13 than known
     commercial antibodies (Figure 7).

15   In addition to antibody sequences, a specific binding member
     according to the present invention may comprise other amino
     acids, e.g. forming a peptide or polypeptide, such as a folded
     domain, or to impart to the molecule another functional
     characteristic in addition to ability to bind antigen.
20   Specific binding members of the invention may carry a
     detectable label, or may be conjugated to a toxin or a
     targeting moiety or enzyme (e.g. via a peptidyl bond or
     linker).

25   In further aspects, the invention provides an isolated nucleic
     acid which comprises a sequence encoding a specific binding
     member, VH domain and/or VL domains according to the present
     invention, and methods of preparing a specific binding member,
     a VH domain and/or a VL domain of the invention, which
30   comprise expressing said nucleic acid under conditions to
     bring about production of said specific binding member, VH
     domain and/or VL domain, and recovering it.

Specific binding members according to the invention may be
used in a method of treatment or diagnosis of the human or
animal body, such as a method of treatment (which may include
prophylactic treatment) of a disease or disorder in a human

5   patient which comprises administering to said patient an
effective amount of a specific binding member of the
invention. Conditions treatable in accordance with the
present invention include any in which IL-13 plays a role,
especially asthma, atopic dermatitis, allergic rhinitis,

10   fibrosis, chronic obstructive pulmonary disease, scleroderma,
inflammatory bowel disease and Hodgkin's lymphoma. Further,
the antibodies of the present invention may also be used in
treating tumours and viral infections as these antibodies will
inhibit IL-13 mediated immunosupression [64, 65].

15

A further aspect of the present invention provides nucleic
acid, generally isolated, encoding an antibody VH variable
domain and/or VL variable domain disclosed herein.

20   Another aspect of the present invention provides nucleic acid,
generally isolated, encoding a VH CDR or VL CDR sequence
disclosed herein, especially a VH CDR selected from SEQ ID
NO's: 7, 8 and 9 or a VL CDR selected from SEQ ID NO's: 10, 11
and 12, most preferably BAK502G9 VH CDR3 (SEQ ID NO: 9).

25   Nucleic acid encoding the BAK502G9 set of CDR's, nucleic acid
encoding the BAK502G9 set of HCDR's and nucleic acid encoding
the BAK502G9 set of LCDR's are also provided by the present
invention, as are nucleic acids encoding individual CDR's,
HCDR's, LCDR's and sets of CDR's, HCDR's, LCDR's of the

30   BAK278D6 lineage.

A further aspect provides a host cell transformed with nucleic
acid of the invention.

A yet further aspect provides a method of production of an
antibody VH variable domain, the method including causing
expression from encoding nucleic acid. Such a method may
comprise culturing host cells under conditions for production

5   of said antibody VH variable domain.

Analogous methods for production of VL variable domains and
specific binding members comprising a VH and/or VL domain are
provided as further aspects of the present invention.

10

A method of production may comprise a step of isolation and/or
purification of the product.

A method of production may comprise formulating the product
15   into a composition including at least one additional
component, such as a pharmaceutically acceptable excipient.

These and other aspects of the invention are described in
further detail below.

20

TERMINOLOGY

Specific binding member
This describes a member of a pair of molecules which have
25   binding specificity for one another. The members of a
specific binding pair may be naturally derived or wholly or
partially synthetically produced. One member of the pair of
molecules has an area on its surface, or a cavity, which
specifically binds to and is therefore complementary to a
30   particular spatial and polar organisation of the other member
of the pair of molecules. Thus the members of the pair have
the property of binding specifically to each other. Examples
of types of specific binding pairs are antigen-antibody,
biotin-avidin, hormone-hormone receptor, receptor-ligand,

enzyme-substrate.   The present invention is concerned with
antigen-antibody type reactions.

Antibody molecule

5   This describes an immunoglobulin whether natural or partly or
wholly synthetically produced.   The term also covers any
polypeptide or protein comprising an antibody binding domain.
Antibody fragments which comprise an antigen binding domain
are molecules such as Fab, scFv, Fv, dAb, Fd; and diabodies.

10

It is possible to take monoclonal and other antibodies and use
techniques of recombinant DNA technology to produce other
antibodies or chimeric molecules which retain the specificity
of the original antibody.   Such techniques may involve

15   introducing DNA encoding the immunoglobulin variable region,
or the complementarity determining regions (CDRs), of an
antibody to the constant regions, or constant regions plus
framework regions, of a different immunoglobulin.   See, for
instance, EP-A-184187, GB 2188638A or EP-A-239400, and a large

20   body of subsequent literature.   A hybridoma or other cell
producing an antibody may be subject to genetic mutation or
other changes, which may or may not alter the binding
specificity of antibodies produced.

25   As antibodies can be modified in a number of ways, the term
"antibody molecule" should be construed as covering any
specific binding member or substance having an antibody
antigen-binding domain with the required specificity.   Thus,
this term covers antibody fragments and derivatives, including

30   any polypeptide comprising an immunoglobulin binding domain,
whether natural or wholly or partially synthetic.   Chimeric
molecules comprising an immunoglobulin binding domain, or
equivalent, fused to another polypeptide are therefore
included.   Cloning and expression of chimeric antibodies are

described in EP-A-0120694 and EP-A-0125023, and a large body
of subsequent literature.

Further techniques available in the art of antibody
5   engineering have made it possible to isolate human and
humanised antibodies. For example, human hybridomas can be
made as described by Kontermann et al [107]. Phage display,
another established technique for generating specific binding
members has been described in detail in many publications such
10   as Kontermann et al [107] and WO92/01047 (discussed further
below). Transgenic mice in which the mouse antibody genes are
inactivated and functionally replaced with human antibody
genes while leaving intact other components of the mouse
immune system, can be used for isolating human antibodies to
15   human antigens [108].

Synthetic antibody molecules may be created by expression from
genes generated by means of oligonucleotides synthesized and
assembled within suitable expression vectors, for example as
20   described by Knappik et al. J. Mol. Biol. (2000) 296, 57-86 or
Krebs et al. Journal of Immunological Methods 254 2001 67-84.

It has been shown that fragments of a whole antibody can
perform the function of binding antigens. Examples of binding
25   fragments are (i) the Fab fragment consisting of VL, VH, CL
and CH1 domains; (ii) the Fd fragment consisting of the VH and
CH1 domains; (iii) the Fv fragment consisting of the VL and VH
domains of a single antibody; (iv) the dAb fragment (Ward,
E.S. et al., Nature 341, 544-546 (1989), McCafferty et al
30   (1990) Nature, 348, 552-554) which consists of a VH domain;
(v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent
fragment comprising two linked Fab fragments (vii) single
chain Fv molecules (scFv), wherein a VH domain and a VL domain
are linked by a peptide linker which allows the two domains to

associate to form an antigen binding site (Bird et al,
Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-
5883, 1988); (viii) bispecific single chain Fv dimers
(PCT/US92/09965) and (ix) "diabodies", multivalent or

5   multispecific fragments constructed by gene fusion
(WO94/13804; P. Holliger et al, Proc. Natl. Acad. Sci. USA 90
6444-6448, 1993). Fv, scFv or diabody molecules may be
stabilised by the incorporation of disulphide bridges linking
the VH and VL domains (Y. Reiter et al, Nature Biotech, 14,

10   1239-1245, 1996). Minibodies comprising a scFv joined to a
CH3 domain may also be made (S. Hu et al, Cancer Res., 56,
3055-3061, 1996).

Where bispecific antibodies are to be used, these may be
15   conventional bispecific antibodies, which can be manufactured
in a variety of ways (Holliger, P. and Winter G. Current
Opinion Biotechnol. 4, 446-449 (1993)), e.g. prepared
chemically or from hybrid hybridomas, or may be any of the
bispecific antibody fragments mentioned above. Examples of

20   bispecific antibodies include those of the BiTE™ technology in
which the binding domains of two antibodies with different
specificity can be used and directly linked via short flexible
peptides. This combines two antibodies on a short single
polypeptide chain. Diabodies and scFv can be constructed

25   without an Fc region, using only variable domains, potentially
reducing the effects of anti-idiotypic reaction.

Bispecific diabodies, as opposed to bispecific whole
antibodies, may also be particularly useful because they can
30   be readily constructed and expressed in E.coli. Diabodies
(and many other polypeptides such as antibody fragments) of
appropriate binding specificities can be readily selected
using phage display (WO94/13804) from libraries. If one arm
of the diabody is to be kept constant, for instance, with a

specificity directed against IL-13, then a library can be made
where the other arm is varied and an antibody of appropriate
specificity selected. Bispecific whole antibodies may be made
by knobs-into-holes engineering (J. B. B. Ridgeway et al,
5 Protein Eng., 9, 616-621, 1996).

Antigen-binding domain
This describes the part of an antibody molecule which
comprises the area which specifically binds to and is
10 complementary to part or all of an antigen. Where an antigen
is large, an antibody may only bind to a particular part of
the antigen, which part is termed an epitope. An antigen
binding domain may be provided by one or more antibody
variable domains (e.g. a so-called Fd antibody fragment
15 consisting of a VH domain). Preferably, an antigen binding
domain comprises an antibody light chain variable region (VL)
and an antibody heavy chain variable region (VH).

Specific
20 This may be used to refer to the situation in which one member
of a specific binding pair will not show any significant
binding to molecules other than its specific binding
partner(s). The term is also applicable where e.g. an antigen
binding domain is specific for a particular epitope which is
25 carried by a number of antigens, in which case the specific
binding member carrying the antigen binding domain will be
able to bind to the various antigens carrying the epitope.

Comprise
30 This is generally used in the sense of include, that is to say
permitting the presence of one or more features or components.

Isolated

This refers to the state in which specific binding members of
the invention, or nucleic acid encoding such binding members,
5   will generally be in accordance with the present invention.
Isolated members and isolated nucleic acid will be free or
substantially free of material with which they are naturally
associated such as other polypeptides or nucleic acids with
which they are found in their natural environment, or the
10   environment in which they are prepared (e.g. cell culture)
when such preparation is by recombinant DNA technology
practised *in vitro* or *in vivo*. Members and nucleic acid may
be formulated with diluents or adjuvants and still for
practical purposes be isolated - for example the members will
15   normally be mixed with gelatin or other carriers if used to
coat microtitre plates for use in immunoassays, or will be
mixed with pharmaceutically acceptable carriers or diluents
when used in diagnosis or therapy. Specific binding members
may be glycosylated, either naturally or by systems of
20   heterologous eukaryotic cells (e.g. CHO or NSO (ECACC
85110503) cells, or they may be (for example if produced by
expression in a prokaryotic cell) unglycosylated.

Naturally occurring IL-13

25   This generally refers to a state in which the IL-13 protein or
fragments thereof may occur. Naturally occurring IL-13 means
IL-13 protein which is naturally produced by a cell, without
prior introduction of encoding nucleic acid using recombinant
technology. Thus, naturally occurring IL-13 may be as
30   produced naturally by for example CD4+ T cells and/or as
isolated from a mammal, e.g. human, non-human primate, rodent
such as rat or mouse.

Recombinant IL-13

This refers to a state in which the IL-13 protein or fragments
thereof may occur. Recombinant IL-13 means IL-13 protein or
5   fragments thereof produced by recombinant DNA in a
heterologous host. Recombinant IL-13 may differ from
naturally occurring IL-13 by glycosylation.

Recombinant proteins expressed in prokaryotic bacterial
10   expression systems are not glycosylated while those expressed
in eukaryotic systems such as mammalian or insect cells are
glycosylated. Proteins expressed in insect cells however
differ in glycosylation from proteins expressed in mammalian
cells.
15

By "substantially as set out" it is meant that the relevant
CDR or VH or VL domain of the invention will be either
identical or highly similar to the specified regions of which
the sequence is set out herein. By "highly similar" it is
20   contemplated that from 1 to 5, preferably from 1 to 4 such as
1 to 3 or 1 or 2, or 3 or 4, amino acid substitutions may be
made in the CDR and/or VH or VL domain.

The structure for carrying a CDR or a set of CDR's of the
25   invention will generally be of an antibody heavy or light
chain sequence or substantial portion thereof in which the CDR
or set of CDR's is located at a location corresponding to the
CDR or set of CDR's of naturally occurring VH and VL antibody
variable domains encoded by rearranged immunoglobulin genes.
30   The structures and locations of immunoglobulin variable
domains may be determined by reference to (Kabat, E.A. et al,
Sequences of Proteins of Immunological Interest. 4th Edition.
US Department of Health and Human Services. 1987, and updates
thereof, now available on the Internet

(http://immuno.bme.nwu.edu or find "Kabat" using any search
engine).

CDR's can also be carried by other scaffolds such as
5   fibronectin or cytochrome B [115, 116].

Preferably, a CDR amino acid sequence substantially as set out
herein is carried as a CDR in a human variable domain or a
substantial portion thereof. The HCDR3 sequences
10   substantially as set out herein represent preferred
embodiments of the present invention and it is preferred that
each of these is carried as a HCDR3 in a human heavy chain
variable domain or a substantial portion thereof.

15   Variable domains employed in the invention may be obtained
from any germ-line or rearranged human variable domain, or may
be a synthetic variable domain based on consensus sequences of
known human variable domains. A CDR sequence of the invention
(e.g. CDR3) may be introduced into a repertoire of variable
20   domains lacking a CDR (e.g. CDR3), using recombinant DNA
technology.

For example, Marks et al (Bio/Technology, 1992, 10:779-783)
describe methods of producing repertoires of antibody variable
25   domains in which consensus primers directed at or adjacent to
the 5' end of the variable domain area are used in conjunction
with consensus primers to the third framework region of human
VH genes to provide a repertoire of VH variable domains
lacking a CDR3. Marks et al further describe how this
30   repertoire may be combined with a CDR3 of a particular
antibody. Using analogous techniques, the CDR3-derived
sequences of the present invention may be shuffled with
repertoires of VH or VL domains lacking a CDR3, and the
shuffled complete VH or VL domains combined with a cognate VL

or VH domain to provide specific binding members of the
invention. The repertoire may then be displayed in a suitable
host system such as the phage display system of WO92/01047 or
any of a subsequent large body of literature, including Kay,

5   B.K., Winter, J., and McCafferty, J. (1996) Phage Display of
Peptides and Proteins: A Laboratory Manual, San Diego:
Academic Press, so that suitable specific binding members may
be selected. A repertoire may consist of from anything from
10^4 individual members upwards, for example from 10^6 to 10^8 or

10   10^{10} members. Other suitable host systems include yeast
display, bacterial display, T7 display, ribosome display and
so on. For a review of ribosome display for see Lowe D and
Jermutus L, 2004, Curr. Pharm, Biotech, 517-27, also
WO92/01047.

15

Analogous shuffling or combinatorial techniques are also
disclosed by Stemmer (*Nature*, 1994, <u>370</u>:389-391), who
describes the technique in relation to a β-lactamase gene but
observes that the approach may be used for the generation of

20   antibodies.

A further alternative is to generate novel VH or VL regions
carrying CDR-derived sequences of the invention using random
mutagenesis of one or more selected VH and/or VL genes to

25   generate mutations within the entire variable domain. Such a
technique is described by Gram *et al* (1992, *Proc. Natl. Acad.
Sci., USA,* <u>89</u>:3576-3580), who used error-prone PCR. In
preferred embodiments one or two amino acid substitutions are
made within a set of HCDR's and/or LCDR's.

30

Another method which may be used is to direct mutagenesis to
CDR regions of VH or VL genes. Such techniques are disclosed
by Barbas *et al*, (1994, *Proc. Natl. Acad. Sci., USA,* <u>91</u>:3809-
3813) and Schier *et al* (1996, *J. Mol. Biol.* <u>263</u>:551-567).

All the above described techniques are known as such in the
art and in themselves do not form part of the present
invention. The skilled person will be able to use such
5   techniques to provide specific binding members of the
invention using routine methodology in the art.

A further aspect of the invention provides a method for
obtaining an antibody antigen binding domain specific for IL-
10   13 antigen, the method comprising providing by way of
addition, deletion, substitution or insertion of one or more
amino acids in the amino acid sequence of a VH domain set out
herein a VH domain which is an amino acid sequence variant of
the VH domain, optionally combining the VH domain thus
15   provided with one or more VL domains, and testing the VH
domain or VH/VL combination or combinations to identify a
specific binding member or an antibody antigen binding domain
specific for IL-13 antigen and optionally with one or more
preferred properties, preferably ability to neutralise IL-13
20   activity. Said VL domain may have an amino acid sequence
which is substantially as set out herein.

An analogous method may be employed in which one or more
sequence variants of a VL domain disclosed herein are combined
25   with one or more VH domains.

In a preferred embodiment, BAK502G9 VH domain (SEQ ID NO: 15)
may be subject to mutation to provide one or more VH domain
amino acid sequence variants, and/or BAK502G9 VL (SEQ ID NO:
30   16).

A further aspect of the invention provides a method of
preparing a specific binding member specific for IL-13
antigen, which method comprises:

(a)   providing a starting repertoire of nucleic acids
encoding a VH domain which either include a CDR3 to be
replaced or lack a CDR3 encoding region;

(b)   combining said repertoire with a donor nucleic acid
5   encoding an amino acid sequence substantially as set out
herein for a VH CDR3 such that said donor nucleic acid is
inserted into the CDR3 region in the repertoire, so as to
provide a product repertoire of nucleic acids encoding a VH
domain;

10   (c)   expressing the nucleic acids of said product
repertoire;

(d)   selecting a specific binding member specific for a
IL-13; and

(e)   recovering said specific binding member or nucleic
15   acid encoding it.

Again, an analogous method may be employed in which a VL CDR3
of the invention is combined with a repertoire of nucleic
acids encoding a VL domain which either include a CDR3 to be
20   replaced or lack a CDR3 encoding region.

Similarly, one or more, or all three CDRs may be grafted into
a repertoire of VH or VL domains which are then screened for a
specific binding member or specific binding members specific
25   for IL-13.

In a preferred embodiment, one or more of BAK502G9 HCDR1 (SEQ
ID NO: 7), HCDR2 (SEQ ID NO: 8) and HCDR3 (SEQ ID NO: 9), or
the BAK502G9 set of HCDR's, may be employed, and/or one or
30   more of BAK502G9 LCDR1 (SEQ ID NO: 10), LCDR2 (SEQ ID NO: 11),
or the BAK502G9 set of LCDR's.

A substantial portion of an immunoglobulin variable domain
will comprise at least the three CDR regions, together with

their intervening framework regions. Preferably, the portion
will also include at least about 50% of either or both of the
first and fourth framework regions, the 50% being the C-
terminal 50% of the first framework region and the N-terminal

5   50% of the fourth framework region. Additional residues at
the N-terminal or C-terminal end of the substantial part of
the variable domain may be those not normally associated with
naturally occurring variable domain regions. For example,
construction of specific binding members of the present

10   invention made by recombinant DNA techniques may result in the
introduction of N- or C-terminal residues encoded by linkers
introduced to facilitate cloning or other manipulation steps.
Other manipulation steps include the introduction of linkers
to join variable domains of the invention to further protein

15   sequences including immunoglobulin heavy chains, other
variable domains (for example in the production of diabodies)
or protein labels as discussed in more detail elsewhere
herein.

20   Although in a preferred aspect of the invention specific
binding members comprising a pair of VH and VL domains are
preferred, single binding domains based on either VH or VL
domain sequences form further aspects of the invention. It is
known that single immunoglobulin domains, especially VH

25   domains, are capable of binding target antigens in a specific
manner.

In the case of either of the single specific binding domains,
these domains may be used to screen for complementary domains

30   capable of forming a two-domain specific binding member able
to bind IL-13.

This may be achieved by phage display screening methods using
the so-called hierarchical dual combinatorial approach as

disclosed in WO92/01047, in which an individual colony
containing either an H or L chain clone is used to infect a
complete library of clones encoding the other chain (L or H)
and the resulting two-chain specific binding member is
5   selected in accordance with phage display techniques such as
those described in that reference. This technique is also
disclosed in Marks et al, ibid.

Specific binding members of the present invention may further
10 comprise antibody constant regions or parts thereof. For
example, a VL domain may be attached at its C-terminal end to
antibody light chain constant domains including human Cκ or Cλ
chains, preferably Cλ chains. Similarly, a specific binding
member based on a VH domain may be attached at its C-terminal
15 end to all or part (e.g. a CH1 domain) of an immunoglobulin
heavy chain derived from any antibody isotype, e.g. IgG, IgA,
IgE and IgM and any of the isotype sub-classes, particularly
IgG1 and IgG4. IgG4 is preferred. IgG4 is preferred because
it does not bind complement and does not create effector
20 functions. Any synthetic or other constant region variant
that has these properties and stabilizes variable regions is
also preferred for use in embodiments of the present
invention.

25 Specific binding members of the invention may be labelled with
a detectable or functional label. Detectable labels include
radiolabels such as ^{131}I or ^{99}Tc, which may be attached to
antibodies of the invention using conventional chemistry known
in the art of antibody imaging. Labels also include enzyme
30 labels such as horseradish peroxidase. Labels further include
chemical moieties such as biotin which may be detected via
binding to a specific cognate detectable moiety, e.g. labelled
avidin.

Specific binding members of the present invention are designed
to be used in methods of diagnosis or treatment in human or
animal subjects, preferably human.

5   Accordingly, further aspects of the invention provide methods
     of treatment comprising administration of a specific binding
     member as provided, pharmaceutical compositions comprising
     such a specific binding member, and use of such a specific
     binding member in the manufacture of a medicament for
10   administration, for example in a method of making a medicament
     or pharmaceutical composition comprising formulating the
     specific binding member with a pharmaceutically acceptable
     excipient.

15   Clinical indications in which an anti-IL-13 antibody may be
     used to provide therapeutic benefit include asthma, atopic
     dermatitis, allergic rhinitis, fibrosis, chronic obstructive
     pulmonary disease, inflammatory bowel disease, scleroderma and
     Hodgkin's lymphoma . As already explained, anti-IL-13
20   treatment is effective for all these diseases.

     Anti-IL-13 treatment may be given orally, by injection (for
     example, subcutaneously, intravenously, intraperitoneal or
     intramuscularly), by inhalation, or topically (for example
25   intraocular, intranasal, rectal, into wounds, on skin). The
     route of administration can be determined by the
     physicochemical characteristics of the treatment, by special
     considerations for the disease or by the requirement to
     optimise efficacy or to minimise side-effects.
30
     It is envisaged that anti-IL-13 treatment will not be
     restricted to use in the clinic. Therefore, subcutaneous
     injection using a needle free device is also preferred.

Combination treatments may be used to provide significant
synergistic effects, particularly the combination of an anti-
IL-13 specific binding member with one or more other drugs. A
specific binding member according to the present invention may
5   be provided in combination or addition to short or long acting
beta agonists, corticosteroids, cromoglycate, leukotriene
(receptor) antagonists, methyl xanthines and their
derivatives, IL-4 inhibitors, muscarinic receptor antagonists,
IgE inhibitors, histaminic inhibitors, IL-5 inhibitors,
10   eotaxin/CCR3 inhibitors, PDE4 inhibitors, TGF-beta
antagonists, interferon-gamma, perfenidone, chemotherapeutic
agents and immunotherapeutic agents.

Combination treatment with one or more short or long acting
15   beta agonists, corticosteroids, cromoglycate, leukotriene
(receptor) antagonists, xanthines, IgE inhibitors, IL-4
inhibitors, IL-5 inhibitors, eotaxin/CCR3 inhibitors, PDE4
inhibitors may be employed for treatment of asthma. Antibodies
of the present invention can also be used in combination with
20   corticosteroids, anti-metabolites, antagonists of TGF-beta and
its downstream signalling pathway, for treatment of fibrosis.
Combination therapy of these antibodies with PDE4 inhibitors,
xanthines and their derivatives, muscarinic receptor
antagonists, short and long beta antagonists can be useful for
25   treating chronic obstructive pulmonary disease. Similar
consideration of combinations apply to the use of anti-IL-13
treatment for atopic dermatitis, allergic rhinitis, chronic
obstructive pulmonary disease, inflammatory bowel disease,
scleroderma and Hodgkin's lymphoma.
30

In accordance with the present invention, compositions
provided may be administered to individuals. Administration
is preferably in a "therapeutically effective amount", this
being sufficient to show benefit to a patient. Such benefit

may be at least amelioration of at least one symptom. The
actual amount administered, and rate and time-course of
administration, will depend on the nature and severity of what
is being treated. Prescription of treatment, e.g. decisions
5   on dosage etc, is within the responsibility of general
practitioners and other medical doctors. Appropriate doses of
antibody are well known in the art; see Ledermann J.A. et al.
(1991) Int. J. Cancer 47: 659-664; Bagshawe K.D. et al. (1991)
Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-
10   922.

The precise dose will depend upon a number of factors,
including whether the antibody is for diagnosis or for
treatment, the size and location of the area to be treated,
15   the precise nature of the antibody (e.g. whole antibody,
fragment or diabody), and the nature of any detectable label
or other molecule attached to the antibody. A typical
antibody dose will be in the range 100µg to 1 gm for systemic
applications, and 1µg to 1mg for topical applications.
20   Typically, the antibody will be a whole antibody, preferably
the IgG4 isotype. This is a dose for a single treatment of an
adult patient, which may be proportionally adjusted for
children and infants, and also adjusted for other antibody
formats in proportion to molecular weight. Treatments may be
25   repeated at daily, twice-weekly, weekly or monthly intervals,
at the discretion of the physician. In preferred embodiments
of the present invention, treatment is periodic, and the
period between administrations is about two weeks or more,
preferably about three weeks or more, more preferably about
30   four weeks or more, or about once a month.

Specific binding members of the present invention will usually
be administered in the form of a pharmaceutical composition,

which may comprise at least one component in addition to the specific binding member.

Thus pharmaceutical compositions according to the present
5   invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not
10   interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. intravenous.

15   Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils,
20   mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

25   For intravenous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable
30   solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

5   Specific binding members of the present invention may be formulated in liquid or solid forms depending on the physicochemical properties of the molecule and the route of delivery. Formulations may include excipients, or combinations of excipients, for example: sugars, amino acids and
10   surfactants. Liquid formulations may include a wide range of antibody concentrations and pH. Solid formulations may be produced by lyophilisation, spray drying, or drying by supercritical fluid technology, for example. Formulations of anti-IL-13 will depend upon the intended route of delivery:
15   for example, formulations for pulmonary delivery may consist of particles with physical properties that ensure penetration into the deep lung upon inhalation; topical formulations may include viscosity modifying agents, which prolong the time that the drug is resident at the site of action.
20
       The present invention provides a method comprising causing or allowing binding of a specific binding member as provided herein to IL-13. As noted, such binding may take place *in vivo*, e.g. following administration of a specific binding
25   member, or nucleic acid encoding a specific binding member, or it may take place *in vitro*, for example in ELISA, Western blotting, immunocytochemistry, immuno-precipitation, affinity chromatography, or cell based assays such as a TF-1 assay.

30   The amount of binding of specific binding member to IL-13 may be determined. Quantitation may be related to the amount of the antigen in a test sample, which may be of diagnostic interest.

A kit comprising a specific binding member or antibody
molecule according to any aspect or embodiment of the present
invention is also provided as an aspect of the present
invention. In a kit of the invention, the specific binding

5   member or antibody molecule may be labelled to allow its
reactivity in a sample to be determined, e.g. as described
further below. Components of a kit are generally sterile and
in sealed vials or other containers. Kits may be employed in
diagnostic analysis or other methods for which antibody

10 molecules are useful. A kit may contain instructions for use
of the components in a method, e.g. a method in accordance
with the present invention. Ancillary materials to assist in
or to enable performing such a method may be included within a
kit of the invention.

15

The reactivities of antibodies in a sample may be determined
by any appropriate means. Radioimmunoassay (RIA) is one
possibility. Radioactive labelled antigen is mixed with
unlabelled antigen (the test sample) and allowed to bind to

20 the antibody. Bound antigen is physically separated from
unbound antigen and the amount of radioactive antigen bound to
the antibody determined. The more antigen there is in the
test sample the less radioactive antigen will bind to the
antibody. A competitive binding assay may also be used with

25 non-radioactive antigen, using antigen or an analogue linked
to a reporter molecule. The reporter molecule may be a
fluorochrome, phosphor or laser dye with spectrally isolated
absorption or emission characteristics. Suitable
fluorochromes include fluorescein, rhodamine, phycoerythrin

30 and Texas Red. Suitable chromogenic dyes include
diaminobenzidine.

Other reporters include macromolecular colloidal particles or
particulate material such as latex beads that are coloured,

magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which

5   catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities

10   used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

The signals generated by individual antibody-reporter

15   conjugates may be used to derive quantifiable absolute or relative data of the relevant antibody binding in samples (normal and test).

The present invention also provides the use of a specific

20   binding member as above for measuring antigen levels in a competition assay, that is to say a method of measuring the level of antigen in a sample by employing a specific binding member as provided by the present invention in a competition assay. This may be where the physical separation of bound

25   from unbound antigen is not required. Linking a reporter molecule to the specific binding member so that a physical or optical change occurs on binding is one possibility. The reporter molecule may directly or indirectly generate detectable, and preferably measurable, signals. The linkage

30   of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

The present invention also provides for measuring levels of
antigen directly, by employing a specific binding member
according to the invention for example in a biosensor system.

5

The mode of determining binding is not a feature of the
present invention and those skilled in the art are able to
choose a suitable mode according to their preference and
general knowledge.

10

As noted, in various aspects and embodiments, the present
invention extends to a specific binding member which competes
for binding to IL-13 with any specific binding member defined
herein, e.g. BAK502G9 IgG4. Competition between binding

15 members may be assayed easily *in vitro*, for example by tagging
a specific reporter molecule to one binding member which can
be detected in the presence of other untagged binding
member(s), to enable identification of specific binding
members which bind the same epitope or an overlapping epitope.

20

Competition may be determined for example using ELISA in which
IL-13 is immobilised to a plate and a first tagged binding
member along with one or more other untagged binding members
is added to the plate. Presence of an untagged binding member

25 that competes with the tagged binding member is observed by a
decrease in the signal emitted by the tagged binding member.

In testing for competition a peptide fragment of the antigen
may be employed, especially a peptide including an epitope of

30 interest. A peptide having the epitope sequence plus one or
more amino acids at either end may be used. Such a peptide
may be said to "consist essentially" of the specified
sequence. Specific binding members according to the present
invention may be such that their binding for antigen is

inhibited by a peptide with or including the sequence given.
In testing for this, a peptide with either sequence plus one
or more amino acids may be used.

5

Specific binding members which bind a specific peptide may be
isolated for example from a phage display library by panning
with the peptide(s).

10   The present invention further provides an isolated nucleic
acid encoding a specific binding member of the present
invention. Nucleic acid may include DNA and/or RNA. In a
preferred aspect, the present invention provides a nucleic
acid which codes for a CDR or set of CDR's or VH domain or VL
15   domain or antibody antigen-binding site or antibody molecule,
e.g. scFv or IgG4, of the invention as defined above.

The present invention also provides constructs in the form of
plasmids, vectors, transcription or expression cassettes which
20   comprise at least one polynucleotide as above.

The present invention also provides a recombinant host cell
which comprises one or more constructs as above. A nucleic
acid encoding any CDR or set of CDR's or VH domain or VL
25   domain or antibody antigen-binding site or antibody molecule,
e.g. scFv or IgG4 as provided, itself forms an aspect of the
present invention, as does a method of production of the
encoded product, which method comprises expression from
encoding nucleic acid therefor. Expression may conveniently
30   be achieved by culturing under appropriate conditions
recombinant host cells containing the nucleic acid. Following
production by expression a VH or VL domain, or specific
binding member may be isolated and/or purified using any
suitable technique, then used as appropriate.

Specific binding members, VH and/or VL domains, and encoding
nucleic acid molecules and vectors according to the present
invention may be provided isolated and/or purified, e.g. from
5   their natural environment, in substantially pure or
homogeneous form, or, in the case of nucleic acid, free or
substantially free of nucleic acid or genes origin other than
the sequence encoding a polypeptide with the required
function. Nucleic acid according to the present invention may
10   comprise DNA or RNA and may be wholly or partially synthetic.
Reference to a nucleotide sequence as set out herein
encompasses a DNA molecule with the specified sequence, and
encompasses a RNA molecule with the specified sequence in
which U is substituted for T, unless context requires
15   otherwise.

Systems for cloning and expression of a polypeptide in a
variety of different host cells are well known. Suitable host
cells include bacteria, mammalian cells, plant cells, yeast
20   and baculovirus systems and transgenic plants and animals.
Mammalian cell lines available in the art for expression of a
heterologous polypeptide include Chinese hamster ovary (CHO)
cells, HeLa cells, baby hamster kidney cells, NS0 mouse
melanoma cells, YB2/0 rat myeloma cells, human embryonic
25   kidney cells, human embryonic retina cells and many others. A
common, preferred bacterial host is *E. coli*.

The expression of antibodies and antibody fragments in
prokaryotic cells such as *E. coli* is well established in the
30   art. For a review, see for example Plückthun, A.
Bio/Technology 9: 545-551 (1991). Expression in eukaryotic
cells in culture is also available to those skilled in the art
as an option for production of a specific binding memberfor
example Chadd HE and Chamow SM (2001) 110 Current Opinion in

Biotechnology 12: 188-194, Andersen DC and Krummen L (2002)
Current Opinion in Biotechnology 13: 117, Larrick JW and
Thomas DW (2001) Current opinion in Biotechnology 12:411-418.

5   Suitable vectors can be chosen or constructed, containing
     appropriate regulatory sequences, including promoter
     sequences, terminator sequences, polyadenylation sequences,
     enhancer sequences, marker genes and other sequences as
     appropriate. Vectors may be plasmids, viral e.g. 'phage, or
10   phagemid, as appropriate. For further details see, for
     example, *Molecular Cloning: a Laboratory Manual*: 3rd edition,
     Sambrook and Russell, 2001, Cold Spring Harbor Laboratory
     Press. Many known techniques and protocols for manipulation
     of nucleic acid, for example in preparation of nucleic acid
15   constructs, mutagenesis, sequencing, introduction of DNA into
     cells and gene expression, and analysis of proteins, are
     described in detail in *Current Protocols in Molecular Biology*,
     Second Edition, Ausubel et al. eds., John Wiley & Sons, 1988,
     Short Protocols in Molecular Biology: A Compendium of Methods
20   *from Current Protocols in Molecular Biology*, Ausubel et al.
     eds., John Wiley & Sons, 4th edition 1999. The disclosures of
     Sambrook et al. and Ausubel et al. (both) are incorporated
     herein by reference.

25   Thus, a further aspect of the present invention provides a
     host cell containing nucleic acid as disclosed herein. Such a
     host cell may be *in vitro* and may be in culture. Such a host
     cell may be *in vivo*. *In vivo* presence of the host cell may
     allow intracellular expression of the specific binding members
30   of the present invention as "intrabodies" or intracellular
     antibodies. Intrabodies may be used for gene therapy [112].

     A still further aspect provides a method comprising
     introducing such nucleic acid into a host cell. The

introduction may employ any available technique.   For
eukaryotic cells, suitable techniques may include calcium
phosphate transfection, DEAE-Dextran, electroporation,
liposome-mediated transfection and transduction using

5   retrovirus or other virus, e.g. vaccinia or, for insect cells,
baculovirus. Introducing nucleic acid in the host cell, in
particular a eukaryotic cell may use a viral or a plasmid
based system. The plasmid system may be maintained episomally
or may incorporated into the host cell or into an artificial

10   chromosome [110,111]. Incorporation may be either by random or
targeted integration of one or more copies at single or
multiple loci. For bacterial cells, suitable techniques may
include calcium chloride transformation, electroporation and
transfection using bacteriophage.

15

The introduction may be followed by causing or allowing
expression from the nucleic acid, e.g. by culturing host cells
under conditions for expression of the gene.

20   In one embodiment, the nucleic acid of the invention is
integrated into the genome (e.g. chromosome) of the host cell.
Integration may be promoted by inclusion of sequences which
promote recombination with the genome, in accordance with
standard techniques.

25

The present invention also provides a method which comprises
using a construct as stated above in an expression system in
order to express a specific binding member or polypeptide as
above.

30

Aspects and embodiments of the present invention will now be
illustrated by way of example with reference to the following
experimentation.

EXAMPLE 1

Isolation of anti-IL-13 scFv

ScFv antibody repertoire

5   A large single chain Fv (scFv) human antibody library derived
from spleen lymphocytes from 20 donors and cloned into a
phagemid vector was used for selections [66].

Selection of scFv

10 ScFv which recognised IL-13 were isolated from phage display
libraries in a series of repeated selection cycles on
recombinant bacterially derived human or murine IL-13
(Peprotech) essentially as described in [67]. In brief,
following incubation with the library, the immobilised

15 antigen, which had been pre-coupled to paramagnetic beads, and
bound phage were recovered by magnetic separation whilst
unbound phage were washed away. Bound phage was then rescued
as described by Vaughan et al [67] and the selection process
repeated. Different solid surfaces and capture methods were

20 used at different rounds of selection to reduce non-specific
binding. Antigen was either covalently coupled to beads
(Dynabeads M-270 carboxylic acid) or modified by biotinylation
prior to secondary capture by streptavidin-coated beads
(Dynabeads M-280) according to manufacturer's protocols

25 (Dynal). A representative proportion of clones from the output
of selection rounds were subjected to DNA sequencing as
described in Vaughan et al [67] and Osbourn et al [70].
Unique clones were assessed for their ability to neutralise
IL-13 as purified scFv preparations in IL-13 dependent cell

30 proliferation assays.

Ribosome display libraries were created and screened for scFv
that specifically recognised recombinant, bacterially derived
human or murine IL-13 (Peprotech), essentially as described in

Hanes *et al* [113]. Initially the BAK278D6 lead clone from the
initial selections was converted to ribosome display format,
and this template was subsequently used for library creation.
On the DNA level, a T7 promoter was added at the 5'-end for
5   efficient transcription to mRNA. On the mRNA level, the
construct contained a prokaryotic ribosome-binding site
(Shine-Dalgarno sequence). At the 3' end of the single chain,
the stop codon was removed and a portion of gIII (gene III)
was added to act as a spacer [113].
10

Ribosome display libraries derived from BAK278D6 were created
by mutagenesis of antibody complementarity determining regions
(CDRs) where PCR reactions were performed with non-proof
reading *Taq* polymerase. Affinity-based selections were
15 performed whereby, following incubation with the library, the
biotinylated human-IL-13 was captured by streptavidin-coated
paramagnetic beads (Dynal M280) and bound tertiary complexes
(mRNA-ribosome-scFv-IL-13) were recovered by magnetic
separation whilst unbound complexes were washed away. The
20 mRNA encoding the bound scFvs were then recovered by RT-PCR as
described in Hanes *et al* [113] and the selection process
repeated with decreasing concentrations (100nM - 100pM over 5
rounds) of biotinylated human IL-13 present during the
selection.
25

Error-prone PCR was also used to further increase library
size. Three intensities of error were employed (2.0, 3.5 and
7.2 mutations per 1,000 bp after a standard PCR reaction, as
described in manufacturer's protocol (Clontech)) during the
30 selection regime. Initial error prone PCR reactions took
place before round one selections commenced at 100nM. A
subsequent round of error prone PCR was performed before round
three selections at 10nM biotinylated human-IL-13. As above, a
representative proportion of clones from the output of

selection rounds were subjected to DNA sequencing as described
in Vaughan et al [67] and Osbourn et al [70]. Unique clones
were assessed for their ability to neutralise IL-13 as
purified scFv preparations in IL-13 dependent cell
5   proliferation assays.

EXAMPLE 2
Neutralisation potency of anti-IL-13 scFv in the IL-13
dependent TF-1 cell proliferation assay
10

The neutralisation potency of purified scFv preparations
against human and murine IL-13 bioactivity was assessed using
TF-1 cell proliferation assay. Purified scFv preparations were
prepared as described in Example 3 of WO01/66754. Protein
15   concentrations of purified scFv preparations were determined
using the BCA method (Pierce). TF-1 is a human premyeloid cell
line established from a patient with erythroleukemia [68]. The
TF-1 cell line is factor dependent for survival and
proliferation. In this respect TF-1 cells responded to either
20   human or murine IL-13 [69] and were maintained in media
containing human GM-CSF (4 ng/ml, R&D Systems). Inhibition of
IL-13 dependent proliferation was determined by measuring the
reduction in incorporation of tritiated thymidine into the
newly synthesized DNA of dividing cells.
25

TF-1 cell assay protocol
TF-1 cells were obtained from R&D Systems and maintained
according to supplied protocols. Assay media comprised RPMI-
1640 with GLUTAMAX I (Invitrogen) containing 5% foetal bovine
30   serum (JRH) and 1% sodium pyruvate (Sigma). Prior to each
assay, TF-1 cells were pelleted by centrifugation at 300 x *g*
for 5 mins, the media removed by aspiration and the cells
resuspended in assay media. This process was repeated twice
with cells resuspended at a final concentration of 10^5 cells/ml

in assay media. Test solutions of antibody (in triplicate)
were diluted to the desired concentration in assay media. An
irrelevant antibody not directed at IL-13 was used as a
negative control. Recombinant bacterially derived human or
5   murine IL-13 (Peprotech) was added to a final concentration of
50 ng/ml when mixed with the appropriate test antibody in a
total volume of 100 µl/well in a 96 well assay plate. The
concentration of IL-13 used in the assay was selected as the
dose that at final assay concentration gave approximately 80%
10   of the maximal proliferative response. All samples were
incubated for 30 minutes at room temperature. 100 µl of
resuspended cells were then added to each assay point to give
a total assay volume of 200 µl/well. Assay plates were
incubated for 72 hours at 37°C under 5% CO_2. 25 µl of tritiated
15   thymidine (10 µCi/ml, NEN) was then added to each assay point
and assay plates were returned to the incubator for a further
4 hours. Cells were harvested on glass fibre filter plates
(Perkin Elmer) using a cell harvester. Thymidine incorporation
was determined using a Packard TopCount microplate liquid
20   scintillation counter. Data were analysed using Graphpad Prism
software.

Results
Despite alternating selection cycles between human and murine
25   antigen no cross-reactive neutralising antibodies were
obtained. Two distinct anti-human and one anti-murine IL-13
neutralising scFvs were obtained from selections. BAK278D6 (VH
SEQ ID NO: 13; VL SEQ ID NO: 14) and BAK167A11 (VH SEQ ID NO:
23; VL SEQ ID NO: 24) recognised human IL-13 whilst BAK209B11
30   (VH SEQ ID NO: 25; VL SEQ ID NO: 26) recognised murine IL-13.
BAK278D6 (Figure 2) and BAK167A11 (Figure 1) as scFv
neutralised 25 ng/ml human IL-13 with an IC_{50} of 44nM and 111nM

respectively. BAK209B11 (Figure 3) as a scFv neutralised 25 ng/ml murine IL-13 with an IC_{50} of 185nM.

EXAMPLE 3

5 *Neutralisation potency of lead clones from targeted optimisation of heavy chain CDR3 of parental clones in the IL-13 dependent TF-1 cell proliferation assay*

Osbourn et al. [70] have demonstrated that targeted
10 mutagenesis of residues within heavy chain CDR3 can significantly improve the affinity of antibodies. Selections were performed as described in Example 1, on scFv repertoires in which residues within the heavy chain CDR3 of BAK278D6 (SEQ ID NO: 6) BAK167A11 (SEQ ID NO: 57) had been randomised by
15 mutagenesis. Unique clones from the selection output were identified by DNA sequencing and their neutralising potency assessed as scFv in the TF-1 cell proliferation assay, as described in Example 2.

20 *Results*
Significant gains in potency were achieved for both lineages. The most potent clones from the BAK167A11 lineage were BAK615E3, BAK612B5 and BAK582F7 which as scFv had IC_{50} of 3nM (Figure 1), 6.6nM, 6.65nM respectively against 25ng/ml human
25 IL-13 in TF-1 cell proliferation assay. From the BAK278D6 lineage, the most potent clone was BAK502G9, which as scFv had IC_{50} of 8nM against 25 ng/ml human IL-13 in the TF-1 cell proliferation assay (Figure 2).

30 *EXAMPLE 4*
Neutralisation potency of BAK167A11 and BAK278D6 lineages against non-human primate IL-13 and an IL-13 variant associated with asthma in the TF-1 factor dependent cell proliferation assay

Neither of the BAK167A11 and BAK278D6 human IL-13 neutralising
lineages were murine cross-reactive. The inventors therefore
decided on the following criteria for the lineage selected for
5   further optimisation and clinical development: should
preferably be cross-reactive with non-human primate IL-13 and
should recognise a variant of IL-13, in which arginine at
amino acid at position 130 is substituted for by glutamine
(Q130R). This variant has been genetically associated with
10   asthma and other allergic diseases [37, 39, 41, 71]. Cross-
reactivity was determined by the ability of purified scFv
preparations to bind non-human primate IL-13 and IL-13 variant
by surface plasmon resonance (BIAcore) analysis. Functional
activity was determined using the TF-1 cell proliferation
15   assay.

Production of wild-type, variant and non-human primate IL-13
A cDNA for wild-type human IL-13 was obtained from InvivoGen
and modified by site-directed mutagenesis (Stratagene
20   Quikchange® kit) to yield a cDNA encoding variant IL-13. The
coding sequence for both rhesus and cynomolgus monkey IL-13
was obtained by PCR on genomic DNA template using degenerate
primers based on the human IL-13 sequence. Both non-human
primate (rhesus and cynomolgus) sequences were identical to
25   each other but differed from human IL-13 by seven amino acids
(Figure 19). Recombinant wild type, variant and non-human
primate IL-13 were subsequently expressed using the
baculovirus expression system (Invitrogen). Expression
constructs added a carboxyl terminus affinity tag to the
30   expressed protein that allowed purification from insect cell
conditioned media to near homogeneity.

Qualitative binding assay using BIAcore
The binding affinity of purified scFv preparations to non-

human primate, variant and wild type IL-13 was determined by
surface plasmon resonance measurements using a BIAcore 2000
Biosensor (BIAcore AB) as described in Karlsson et al [72]. In
brief, IL-13 was coupled to CM5 sensorchips using an amine
5   coupling kit (BIAcore) at a surface density of approximately
200Ru and three concentrations of test scFv (approximately
350nM, 175nM and 88nM) in HBS-EP buffer passed over the sensor
chip surface. The resulting sensorgrams were evaluated using
BIA evaluation 3.1 software to provide relative binding data.
10

TF-1 assay protocol

The assay was performed essentially as described in Example 2
with the following modifications: non-human primate IL-13,
15   human variant IL-13 (Q130R) and wild type human IL-13 were
used at concentrations of 50 ng/ml, 25 ng/ml and 25 ng/ml
respectively.

Results
20   BIAcore binding assay data suggested that BAK278D6 but not
BAK167A11 lineage had the required cross-reactivity profile
for further therapeutic development (Table 2). This finding
was supported by bioassay data demonstrating that BAK278D6
(Figure 4) and BAK502G9 (Figure 6) were able to neutralise
25   human IL-13, the human IL-13 (Q130R) variant and non-human
primate IL-13 in the TF-1 cell proliferation assay with near
equivalent potency. In contrast, although BAK615E3 (VH SEQ ID
NO: 33; VL SEQ ID NO: 34) had a significantly increased
potency against human IL-13 over its parent BAK167A11 (VH SEQ
30   ID NO: 23; VL SEQ ID NO: 24) in the TF-1 cell proliferation
assay (Figure 1), neither clone bound non-human primate or
variant IL-13 in the BIAcore binding assay.

Germlining framework regions of BAK278D6 and BAK502G9

The derived amino acid sequence of BAK278D6 VH (SEQ ID NO: 13) and VL (SEQ ID NO: 14) were aligned to the known human germline sequences in the VBASE database [73] and the closest germline identified by sequence similarity. The closest

5   germline for the VH domain of BAK278D6 (SEQ ID NO: 14) and its derivatives, was identified as DP14, a member of the VH1 family. The BAK278D6 VH has 9 changes from the DP14 germline within framework regions. The closest germline for the VL of BAK278D6 was identified as $V_\lambda 3$ 3h. The BAK278D6 VL domain (SEQ

10 ID NO: 14) has only 5 changes from the germline within framework regions. Framework regions of BAK278D6 and its derivatives were returned to germline by site directed mutagenesis (Stratagene Quikchange kit) to identically match native human antibodies.

15

EXAMPLE 5
Neutralisation potency of lead clones from targeted
optimisation of heavy chain CDR1 and heavy chain CDR2
sequences of BAK502G9 in the human IL-13 dependent TF-1 cell

20 *proliferation assay*

A second phase of optimisation was performed using BAK502G9 sequence, with germlined framework regions, as a template. Selections were performed essentially as described in Example

25 1 on scFv repertories in which either residues within the heavy chain CDR1 or heavy chain CDR2 of BAK502G9 had been randomised by mutagenesis. Unique clones from the selection output were identified by DNA sequencing and their neutralising potency assessed as purified scFv preparations in

30 the TF-1 cell proliferation assay as described in Example 2. Vectors were constructed for the most potent scFv clones to allow re-expression as whole human IgG4 antibody as described by Persic et al. (1997 Gene 187; 9-18) with a few modifications. An oriP fragment was included in the vectors to

facilitate use with HEK-EBNA 293 cells and to allow episomal replication. The VH variable domain was cloned into the polylinker between the secretion leader sequence and the human gamma 4 constant domain of the expression vector pEU8.1(+).

5   The VL variable domain was cloned into the polylinker between the secretion leader sequence and the human lambda constant domain of the expression vector pEU4.1(-).

Whole antibody was purified from conditioned media from EBNA-
10 293 cells co-transfected with constructs expressing heavy and light chains by protein A affinity chromatography (Amersham Pharmacia). The purified antibody preparations were sterile filtered and stored at 4°C in phosphate buffered saline (PBS) prior to evaluation. Protein concentration was determined by
15 measuring absorbance at 280nm using the BCA method (Pierce). Reformatted human IgG4 whole antibodies were compared to commercially available anti-human IL-13 antibodies in the TF-1 proliferation assay described in Example 2.

20 *Results*
As demonstrated in Figure 5, the commercial antibody B-B13, (mouse IgG1 -Euroclone 5) was shown to be significantly more potent against human IL-13 than the commercial antibody JES10-5A2 (rat IgG1 - Biosource) with IC_{50} of 1021pM and 471pM
25 respectively. Eight clones, namely, BAK1111D10, BAK1166G02, BAK1167F02, BAK1167F04, BAK1183H4, BAK1184C8, BAK1185E1, BAK1185F8, derived from BAK502G9 (and so "BAK502G9 lineage"), in which the heavy chain CDR1 or CDR2 had been targeted, showed improved potency as scFv over the commercial
30 antibodies. These improvements were maintained on conversion to whole antibody human IgG4. Each of these VH and VL domains individually and in the respective pairings of these claims represents an aspect or embodiment of the present invention, as do specific binding members for IL-13 that comprise one or

more of them, also specific binding members comprising one or more CDR's from the BAK502G9 lineage clones, preferably a VH domain comprising a BAK502G9 lineage set of HCDR's and/or a VL domain comprising a BAK502G9 lineage set of LCDR's. These may

5   be employed in any and all aspects of the invention as disclosed elsewhere herein. Derivatives of BAK502G9 as whole antibodies (IgG4) had an IC_{50} ranging from 244pM to 283pM. BAK502G9 as a whole antibody IgG4 had an IC_{50} of 384pM. In summary, major improvements in potency could be obtained by

10   targeting heavy chain CDR1 (SEQ ID NO:7) or CDR2 (SEQ ID NO: 8) of BAK502G9. Statistical comparisons to B-B13 were made using an ANOVA followed by a Dunnett's post test analysis (InStat software).

15   *Further characterisation*
     Selected anti-human antibodies from the BAK278D6 lineage underwent further characterisation to determine their specificity. These included BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16) and its derivatives BAK1167F2 (VH SEQ ID NO: 35; VL

20   SEQ ID NO: 36) and BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), which are representative examples of clones with modifications to heavy chain CDR1 and heavy chain CDR2 of BAK502G9 respectively.

25   *EXAMPLE 6*
     Neutralisation potency of lead clones from targeted optimisation of heavy chain CDR1 and heavy chain CDR2 sequences of BAK502G9 against non-human primate IL-13 and an IL-13 variant associated with asthma in the TF-1 factor

30   *dependent cell proliferation assay*

     Cross-reactivity of anti-human IL-13 antibodies was determined by their ability to inhibit non-human primate IL-13 and IL-13

variant mediated TF-1 cell proliferation as described in
Example 4.

Results

5 Optimised anti-human IL-13 antibodies BAK1167F2 (VH SEQ ID NO:
35; VL SEQ ID NO: 36) and BAK1183H4 (VH SEQ ID NO: 37; VL SEQ
ID NO: 38) maintained the specificity of their parent BAK502G9
(VH SEQ ID NO: 15; VL SEQ ID NO: 16) (Figure 6). Potency gains
against wild type IL-13 were reflected in their ability to

10 neutralise non-human primate IL-13 and an IL-13 variant with
substantially equivalent potency. The IC_{50} for BAK502G9 against
human, human variant and non-human primate IL-13 were 1.4nM,
1.9nM and 2.0nM respectively. The IC_{50} for BAK1167F2 against
human, human variant and non-human primate IL-13 were 1.0nM,

15 1.1nM and 1.3nM respectively. The IC_{50} for BAK1183H4 against
human, human variant and non-human primate IL-13 were 0.9nM,
1.0nM and 1.6nM respectively. These clones are suitable for
therapeutic use.

20 *EXAMPLE 7*
Neutralising potency of lead anti-human IL-13 antibodies
against native human IL-13 in HDLM-2 cell proliferation assay

The human IL-13 sequence has four potential N-glycosylation

25 sites. The inventors have demonstrated the ability of BAK278D6
and its derivatives to neutralise recombinant IL-13 expressed
either in bacterial or baculovirus expression systems.
Although, there is evidence that many processing events known
in mammalian systems do also occur in insects there are key

30 differences in protein glycosylation, particularly N-
glycosylation [74].

The inventors investigated the ability of BAK278D6 derivatives
to neutralise native IL-13 released from human cells.

HDLM-2 cells were isolated by Drexler et al [75] from a
patient with Hodgkin's disease. Skinnider et al [76]
demonstrated that HDLM-2 cell proliferation was in part
5   dependent on autocrine and paracrine release of IL-13. Lead
anti-human IL-13 antibodies were assessed for their ability to
inhibit HDLM-2 cell proliferation mediated by the release of
native (or naturally occurring) IL-13.

10   *HDLM-2 cell assay protocol*
HDLM-2 cells were obtained from the Deutsche Sammlung von
Mikroorganismen und Zellkulturen (DSMZ) and maintained
according to supplied protocols. Assay media comprised RPI-
1640 with Glutamax I (Invitrogen) containing 20% foetal bovine
15   serum. Prior to each assay, the cells were pelleted by
centrifugation at 300x g for 5 min, the media removed by
aspiration and the cells resuspended in fresh media. This
process was repeated three times and the cells were finally
resuspended to a final concentration of 2 x 10^5 cells/ml in
20   assay media. 50µl of resuspended cells were added to each
assay point in a 96 well assay plate. Test solutions of
antibodies (in triplicate) were diluted to the desired
concentration in assay media. An irrelevant isotype antibody
not directed at IL-13 was used as a negative control. The
25   appropriate test antibody in a total volume of 50µl / well
were added to the cells, each assay point giving a total assay
volume of 100µl / well. Assay plates were incubated for 72
hours at 37°C under 5% CO_2. 25 µl of tritiated thymidine (10
µCi/ml, NEN) was then added to each assay point and assay
30   plates were returned to the incubator for a further 4 hours.
Cells were harvested on glass fibre filter plates (Perkin
Elmer) using a cell harvester. Thymidine incorporation was
determined using a Packard TopCount microplate liquid

scintillation counter. Data were analysed using Graphpad Prism software.

Results

5   As demonstrated in Figure 7, BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16), and its derivatives BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38) and BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) were able to cause a dose dependent inhibition of cell proliferation with relative potencies similar to those

10 observed in other bioassays. IC_{50} for BAK502G9, BAK1183H4, BAK1167F2 as human IgG4 were 4.6nM, 3.5nM and 1.1nM respectively. IC_{50} for the commercial antibodies JES10-5A2 and B-B13 were 10.7nM and 16.7nM respectively.

15 *EXAMPLE 8*
    Neutralising potency of lead anti-human IL-13 antibodies
    against IL-13 dependent responses in disease relevant primary
    cells

20 Secondary bioassays were performed using primary cells and readouts more relevant to airway disease. These included eotaxin release from normal human lung fibroblasts (NHLF) and vascular adhesion molecule 1 (VCAM-1) upregulation on the surface of human umbilical vein endothelial cells (HUVEC).

25 Both IL-13 dependent responses could contribute to eosinophil recruitment, a feature of the asthma phenotype [92].

NHLF assay protocol
    IL-13 has been shown to cause eotaxin release from lung

30 fibroblasts[77] [78] [79]. Factor dependent eotaxin release from NHLF was determined by ELISA.

NHLF were obtained from Biowhittaker and maintained according to supplied protocols. Assay media was FGM-2 (Biowhittaker).

Test solutions of antibody (in triplicate) were diluted to the
desired concentration in assay media. An irrelevant antibody
not directed at IL-13 was used as a negative control.
Recombinant bacterially-derived human IL-13 (Peprotech) was
5   subsequently added to a final concentration of 10 ng/ml when
mixed with the appropriate test antibody in a total volume of
200 µl. The concentration of IL-13 used in the assay was
selected as the dose that gave an approximately 80% of the
maximal response. All samples were incubated for 30 minutes at
10   room temperature. Assay samples were then added to NHLF that
had been preseeded at a density of 1×10^4 cells per well in
96-well assay plates. Assay plates were incubated at 37°C for
16-24 hours at 37°C under 5% CO_2. Assay plates were centrifuged
at 300 x *g* for 5 minutes to pellet detached cells. Eotaxin
15   levels in the supernatant were determined by ELISA using
reagents and methods described by the manufacturer (R&D
Systems). Data were analysed using Graphpad Prism software.

Results
20   BAK278D6 lineage clones were able to inhibit human IL-13
dependent eotaxin release from NHLF. Relative potency was
similar to that observed in the TF-1 cell proliferation assay
(Figure 8). BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16),
BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), BAK1167F2 (VH
25   SEQ ID NO: 35; VL SEQ ID NO: 36) had IC_{50} of 207pM, 118pM and
69pM respectively against 10 ng/ml human IL-13. Commercial
antibodies JES10-5A2 and B-B13 had IC_{50} of 623pM and 219pM
respectively.

30   *HUVEC assay protocol*
IL-13 has been shown to upregulate expression of VCAM-1 on
cell surface of HUVECs [80, 81]. Factor dependent VCAM-1
expression was determined by detection of upregulation of

VCAM-1 receptor cellular expression using a time-resolved
fluorescence read out.

HUVEC were obtained from Biowhittaker and maintained according
5   to supplied protocols. Assay media was EGM-2 (Biowhittaker).
Test solutions of antibody (in triplicate) were diluted to the
desired concentration in assay media. An irrelevant antibody
not directed at IL-13 was used as a negative control.
Recombinant bacterially derived human IL-13 (Peprotech) was
10   added to a final concentration of 10 ng/ml when mixed with the
appropriate test antibody in a total volume of 200 µl. The
concentration of IL-13 used in the assay was selected as the
dose that gave approximately 80% of the maximal response. All
samples were incubated for 30 minutes at room temperature.
15   Assay samples were then added to HUVEC that had been preseeded
at 4 x 10^4 cells per well in 96-well assay plates. Assay
plates were incubated at 37°C for 16-20 hours under 5% CO_2.
Assay media was then removed by aspiration and replaced with
blocking solution (PBS containing 4% dried Marvel® milk
20   powder). Assay plates were incubated at room temperature for 1
hour at room temperature. Wells were washed three times with
PBST Tween before 100 µl (1:500 dilution in PBST/1% Marvel®)
of biotinylated anti-VCAM-1 antibody (Serotec) was added to
each well. Assay plates were incubated at room temperature for
25   1 hour. Wells were washed three times with Delfia wash buffer
(Perkin Elmer) before 100 µl of Europium-labelled Streptavidin
or anti-murine IgG1 (1:1000 dilution in Delfia assay buffer,
Perkin Elmer) was added to each well. Assay plates were then
incubated at RT for 1 hour. Wells were washed 7 times with
30   Delfia wash buffer (Perkin Elmer). Finally, 100µl of
enhancement solution (Perkin Elmer) was added to each well and
fluorescence intensity was determined using the Wallac 1420

VICTOR2 plate reader (Standard Europium protocol). Data were
analysed using Graphpad Prism software.

Results

5   Typical data for BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO:
16), BAK1183H4 (VH SEQ ID NO: 37; VL SEQ ID NO: 38), BAK1167F2
(VH SEQ ID NO: 35; VL SEQ ID NO: 36) as whole antibody human
IgG4 are shown in Figure 9. Relative potency was similar to
the observed in the TF-1 cell proliferation assay. IC_{50} for

10 BAK502G9, BAK1183H4 and BAK1167F2 were 235pM, 58pM and 55pM
respectively against 10ng/ml human IL-13.

EXAMPLE 9

Neutralisation potency of anti-IL-13 antibodies against IL-1β

15 *and IL-4 dependent VCAM-1 upregulation*

The specificity of the BAK278D6 lineage of clones was assessed
in a modification of the HUVEC bioassay. Together with IL-13,
both IL-4 and IL-1β have been shown to upregulate expression

20 of VCAM-1 on cell surface of HUVECs [80, 81].

HUVEC assay protocol
The assay was performed essentially as described in Example 5
with the following modifications. Recombinant human IL-1β and

25 IL-4 (R&D Systems) were used in place of human IL-13 at 0.5
ng/ml and 1 ng/ml respectively and represented the dose that
gave approximately 80% of the maximal response.

Results

30 None of the clones evaluated from the BAK278D6 lineage
neutralised VCAM-1 upregulation in response to either human
IL-1β or IL-4 and thus demonstrated specificity for IL-13
(Figure 10). IL-4 is most closely related to IL-13, sharing
30% sequence identity at the amino acid level [82].

EXAMPLE 10

Neutralisation potency of BAK209B11 as a human IgG4 in a
murine IL-13 dependent murine B9 cell proliferation assay

5

BAK209B11, identified as an anti-murine IL-13 neutralising
clone as a scFv as described in Example 1, was reformatted as
a whole antibody human IgG4 as described in Example 5 and its
potency evaluated in the murine IL-13 dependent B9 cell

10   proliferation assay. B9 is a murine B-cell hybridoma cell line
[83]. B9 is factor dependent for survival and proliferation.
In this respect B cells respond to murine IL-13 and are
maintained in media containing human IL-6 (50pg/ml, R&D
Systems). Inhibition of murine IL-13 dependent proliferation

15   was determined by measuring the reduction in incorporation of
tritiated thymidine into the newly synthesized DNA of dividing
cells.

      B9 cell assay protocol

20   B9 cells were obtained from European Collection of Animal Cell
Culture ECACC and maintained according to supplied protocols.
The assay was performed essentially as described for the TF-1
assay in Example 2 but with the following modifications. Assay
media comprised RPMI-1640 with GLUTAMAX I (Invitrogen)

25   containing 5% foetal bovine serum (Hyclone) and 50μM 2-
mercaptoethanol (Invitrogen). Recombinant bacterially derived
murine IL-13 (Peprotech) replaced human IL-13 with a final
assay concentration of 1ng/ml.

30   Results
      BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26) as a human
IgG4 neutralised 1 ng/ml murine IL-13 with an IC_{50} of 776pM in
the B9 assay (Figure 11). BAK209B11 therefore represents a
useful tool to investigate the role of IL-13 in murine models

of disease. This is clearly demonstrated in Example 12, which demonstrates the efficacy of BAK209B11 in a murine model of acute pulmonary inflammation.

5   *EXAMPLE 11*
     Affinity determination of anti-IL-13 antibodies by BIAcore
     analysis

     The affinity of BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16),
10   BAK1167F2 (VH SEQ ID NO: 35; VL SEQ ID NO: 36) and BAK1183H4
     (VH SEQ ID NO: 37; VL SEQ ID NO: 38) for human IL-13 and
     BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26) for murine IL-
     13 as human IgG4 were determined by surface plasmon resonance
     measurements using a BIAcore 2000 Biosensor (BIAcore AB)
15   essentially as described in [72]. In brief, antibodies were
     coupled to CM5 sensorchips using an amine coupling kit
     (BIAcore) at a surface density of approximately 500Ru and a
     serial dilution of IL-13 (between 50nM to 0.78nM) in HBS-EP
     buffer was passed over the sensorchip surface. The resulting
20   sensorgrams were evaluated using BIA evaluation 3.1 software
     to provide kinetic data.

     Results
     BAK502G9, BAK1167F2 and BAK1183H4 IgG4 bound human IL-13 with
25   high affinity with Kd of 178 pM, 136pM and 81pM respectively
     corresponding to their relative potency in cell based assays.
     BAK209B11 bound murine IL-13 with affinity of 5.1nM   (Table
     3).

EXAMPLE 12

Efficacy of BAK209B11 in a murine model of acute allergic
pulmonary inflammation

5

Murine model of acute allergic pulmonary inflammation
The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an
anti-murine IL-13 neutralising human IgG4 antibody, was
investigated in a murine of acute allergic pulmonary

10   inflammation. This model was performed essentially as
described by Riffo-Vasquez et al [84] and is characterised at
its endpoint by increased bronchial alveolar lavage (BAL) IL-
13 (Figure 12), cellular infiltration into the lung and BAL
(Figure 13), increased serum IgE levels and airways

15   hyperresponsiveness (AHR).

Model protocol
Female Balb/C mice (Charles River UK) were treated with either
anti-murine IL-13 antibody BAK209B11 (at 12, 36, 119 or 357 µg

20   doses) or an isotype matched control antibody (357 µg dose).
On days 0 and 7, mice in each group were sensitised by
intraperitoneal injection of 10µg of ovalbumin (Ova) in 0.2 ml
of the vehicle (saline containing 2% Al_2O_3 (Rehydragel) as an
adjuvant). A separate control group of non-sensitised mice

25   received an equal volume of the vehicle. Mice were challenged
with ovalbumin on days 14, 15 and 16. Ovalbumin was diluted to
1% (w/v) in sterile saline prior to nebulisation. All
inhalation challenges were administered in a Plexiglas
exposure chamber. Ova was aerosolised using a deVilbiss

30   Ultraneb 2000 nebuliser (Sunrise Medical) in a series of three
exposures of 20 minutes separated by 1 hour intervals.

BAK209B11 or an irrelevant human IgG4 were administered
intravenously, 1 day prior to first challenge and then 2 hours

prior to each subsequent challenge (4 doses in total). The
model ended at day 17, 24 hours post final challenge. Blood
(serum) and BAL were collected. Serum was assayed for total
IgE. BAL was obtained by injecting 3 aliquots of saline

5   (0.3ml, 0.3ml and 0.4ml) and pooling samples. Total leukocytes
and differential cell counts were obtained from BAL cells.

Results

Ovalbumin challenge of sensitised mice caused a significant

10   ($p < 0.05$) increase in total BAL cell recruitment over non-
sensitised but challenged animals. This recruitment was dose-
dependently inhibited by BAK209B11; significant ($p < 0.05$)
inhibition was seen with $\geq 36\mu g$ BAK209B11, but not control
antibody (Figure 13). Similar effects were also seen on

15   eosinophils (Figure 14) and neutrophils (Figure 15) with
significant ($p < 0.05$) inhibition of cellular influx at a
minimum BAK209B11 dose of $36\mu g$. This inhibition was not seen
with the control antibody. Lymphocytes were also induced in
sensitised but not non-sensitised mice upon challenge. This

20   induction was dose-dependently inhibited by BAK209B11, with
maximal inhibition seen with $36\mu g$ BAK209B11. Control antibody
had no effect (Figure 16). Although monocyte/macrophages were
not induced in sensitised animals when compared to non-
sensitised animals, background levels were depressed by $\geq 36\mu g$

25   BAK209B11, but not by control antibody (Figure 17). Serum IgE
levels were significantly increased in sensitised animals when
compared to non-sensitised after challenge ($p < 0.05$). This
increase was decreased after treatment with $36\mu g$ BAK209B11 but
not by the control antibody.

30

In summary, systemic administration of BAK209B11, a murine IL-
13 neutralising antibody, but not control antibody inhibited
inflammatory cell influx and the upregulation of serum IgE

levels caused by sensitisation and subsequent challenge with
ovalbumin in a murine model of allergic inflammation.

Examples 13 to 20 are prophetic.

5

EXAMPLE 13
Efficacy of BAK209B11 in the Lloyd murine model of acute
pulmonary inflammation

10   *Murine model of acute allergic pulmonary inflammation*
The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an
anti murine IL-13 neutralising antibody, was investigated in a
second murine model of acute allergic pulmonary inflammation.
This model was performed essentially as described by McMillan

15   et al. [85] and is characterised at its endpoint by increased
BAL and lung tissue IL-13, cellular infiltration into the lung
and BAL, increased serum IgE levels and airways
hyperresponsiveness (AHR).

20   *Model protocol*
Female Balb/C mice (Charles River UK) were administered with
various doses of anti-murine IL-13 antibody BAK209B11 or an
isotype matched control antibody, as follows. On days 0 and
12, mice in each group were sensitised (SN) by intraperitoneal

25   injection of 10µg of ovalbumin (Ova) in 0.2 ml of the vehicle
(saline containing 2mg Al(OH)$_3$ as an adjuvant [calculated as
described in Example 12]). A separate control group of non-
sensitised mice (NS) received an equal volume of the vehicle.
Mice were challenged with ovalbumin for 20 minutes on days 19,

30   20, 21, 22, 23 and 24. Ovalbumin was diluted to 5% (w/v) in
saline prior to nebulisation. All inhalation challenges were
administered in a Plexiglas exposure chamber. Ova was
aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise
Medical). On days 18,19,20,21,22,23 and 24 mice were

administered with various intraperitoneal doses (237µg, 23.7µg or 2.37µg; denoted in figure 21 by H,M and L) of anti-murine IL-13 antibody BAK209B11 muIgG1 or an isotype matched control antibody (237µg). Airway function was assessed on days 0 and

5   25 by increasing methacholine challenges and monitored using conscious plethysmography (Buxco). PC_{50} (concentration of methacholine required to increase baseline PenH by 50%) was estimated for individual mice at both day 0 and day 25 from 4 parameter unfixed curve fitting of methacholine dose-response

10   curves.

The model ended at day 25, 24 hours post final challenge. Blood, serum, BAL and lung tissue were collected.

15   *Results*

Lung function was evaluated for individual animals at day 0 (pre-treatment) and at day 25 (post-challenge) and was quantitated by calculating PC_{50} values (concentration of methacholine required to increase baseline PenH by 50%)

20   (Figure 21A). An individuals airways hyperresponsiveness (AHR) was determined by the change in log PC_{50} at day 25 versus day 0 (log day 25 PC_{50} - log day 0 PC_{50}). This delta logPC_{50} was the primary endpoint of the study; PC_{50} data log-transformed because of requirements of endpoint ANOVA.

25   Individual changes were averaged within groups to generate group average delta log PC_{50} (as shown in Figure 21B).

Ovalbumin challenge of sensitised mice caused a significant AHR compared to non-sensitised and challenged mice (p<0.01).

30   BAK209B11 caused a clear and dose-dependent decrease in AHR whereas the control antibody had no effect.

EXAMPLE 14

*Efficacy of BAK209B11 in the Gerard murine model of acute
pulmonary inflammation*

5

Murine model of acute allergic pulmonary inflammation
The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an
anti-murine IL-13 neutralising human IgG4 antibody, was
investigated in a third murine model of acute allergic
10 pulmonary inflammation. This model was performed essentially
as described by Humbles *et al.* [86] and is characterised at
its endpoint by increased BAL and lung tissue IL-13, cellular
infiltration into the lung and BAL, increased serum IgE levels
and airways hyperresponsiveness (AHR).

15

Model protocol
Female Balb/C mice (Charles River UK) were administered with
various doses of anti-murine IL-13 antibody BAK209B11 or an
isotype matched control antibody. On days 0, 7 and 14, mice in
20 each group were sensitised (SN) by intraperitoneal injection
of 10µg of ovalbumin (Ova) in 0.2 ml of the vehicle (saline
containing 1.125mg $Al(OH)_3$ as an adjuvant [calculated as
described in Example 12]). A separate control group of non-
sensitised mice (NS) received an equal volume of the vehicle.
25 Mice were challenged with ovalbumin for 20 minutes on days 21,
22, 23 and 24. Ovalbumin was diluted to 5% (w/v) in saline
prior to nebulisation. All inhalation challenges were
administered in a Plexiglas exposure chamber. Ova was
aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise
30 Medical).

The model ended at day 25, 24 hours post challenge. Blood,
serum, BAL and lung tissue were collected.

EXAMPLE 15

Efficacy of BAK209B11 in the Lloyd chronic model of pulmonary inflammation

5   *Murine model of chronic allergic pulmonary inflammation*

The effect of BAK209B11 (VH SEQ ID NO: 25; VL SEQ ID NO: 26), an anti murine IL-13 neutralising human IgG4 antibody, was investigated in a model of chronic allergic pulmonary inflammation. This model was performed essentially as

10   described by Temelkovski *et al.* [87] and is characterised at its endpoint by cellular infiltration into the lung and BAL, increased serum IgE levels and airways hyperresponsiveness (AHR).

15   *Model protocol*

Female Balb/C mice (Charles River UK) were dosed with various doses of anti-murine IL-13 antibody BAK209B11 or an isotype matched control antibody. On days 0 and 11, mice in each group were sensitised (SN) by intraperitoneal injection of 10µg of

20   ovalbumin (Ova) in 0.2 ml of the vehicle (saline containing 2mg $Al(OH)_3$ as an adjuvant [calculated as described in Example 12]). A separate control group of non-sensitised mice (NS) received an equal volume of the vehicle. Mice were challenged with ovalbumin for 20 minutes on days 18, 19, 20, 21, 22, 23,

25   28, 30, 32, 35, 37, 39, 42, 44, 46, 49 and 51. Ovalbumin was diluted to 5% (w/v) in saline prior to nebulisation. All inhalation challenges were administered in a Plexiglas exposure chamber. Ova was aerosolised using a deVilbiss Ultraneb 2000 nebuliser (Sunrise Medical).

30

The model ended at day 52, 24 hours post challenge. Blood, serum, BAL and lung tissue were collected.

EXAMPLE 16

Efficacy of anti-human IL-13 antibodies against exogenous
human IL-13 administered to the murine air pouch model

5

The effect of anti-human IL-13 antibodies on the pro-
inflammatory action of human IL-13 was investigated in a basic
murine model. This model was performed essentially as
described by Edwards et al [93] and was characterised at its
10   endpoint by cellular infiltration into the airpouch.

Model protocol
An air pouch was created on the back of female Balb/C mice by
subcutaneous injection of 2.5mL of sterile air at day 0. The
15   air pouch was reinflated with another 2.5mL sterile air at day
3. 2μg huIL-13 in 0.75% CMC was injected directly into the
pouch at day 6. 24 hours later the mice were killed and the
air pouch lavaged with 1mL heparinised saline. Antibody
treatments were either given with the huIL-13 (into the pouch)
20   or given systemically.

Results
Human IL-13, injected into the airpouch (i.po.), caused a
significantly increased infiltration of total leukocytes
25   ($p < 0.01$) and eosinophils ($p < 0.01$) at 24 hours post-challenge
versus vehicle (0.75% carboxymethyl cellulose (CMC) in saline
i.po.) treated mice.

Locally administered BAK502G9 (200mg, 20mg or 2mg intrapouch)
30   significantly and dose-dependently inhibited the total
leukocyte ($p < 0.01$) and eosinophil ($p < 0.01$) infiltration into
the air pouch caused by 2μg huIL-13 in 0.75% CMC.

Systemically administered BAK209B11 (30mg/kg, 10mg/kg and
1mg/kg) also signficantly and dose-dependently inhibited the
total leukocyte ($p<0.01$) and eosinophil ($p<0.01$) infiltration
into the air pouch caused by 2μg huIL-13 in 0.75% CMC.

5

EXAMPLE 17
Generation of human IL-13 knock-in / murine IL-13 knock out
transgenic mice for the purposes of evaluating the efficacy of
anti-human IL-13 antibodies in models of pulmonary allergic
10   *inflammation*

The present inventors have generated mice which express human,
rather than murine IL-13 by gene targeting. The mouse IL-13
gene has been replaced from start to stop codon with the
15   relevant portion of the human IL-13 gene. This mouse strain
expresses human IL-13, rather than mouse IL-13, in response to
the same stimuli as in the wild-type mouse, as the endogenous
IL-13 promoter and IL-13 pA tail remaining unchanged. It has
been shown that human IL-13 can bind to and signal through
20   mouse IL-13 receptors to generate the same physiological
consequences as signalling caused by mouse IL-13 ligating
mouse IL-13 receptors. For example exogenous human IL-13
caused inflammatory cell recruitment into the murine air pouch
(Figure 18). These transgenic animals allow us to evaluate
25   non-murine cross reactive anti-human IL-13 antibodies in
established murine models of disease.

This mouse has been used in the acute allergic airway
inflammation models (as described in examples 18 and 19) and
30   chronic allergic airway inflammation models (as described in
Example 20) allowing the evaluation of anti-human IL-13
antibody pharmacology in allergic airway disease.

EXAMPLE 18
Efficacy of anti-human IL-13 antibodies in the huIL-13-
transgenic Lloyd murine model of acute pulmonary inflammation

5

Murine model of acute allergic pulmonary inflammation
The effect of anti human IL-13 neutralising human IgG4
antibodies were investigated in a murine model of acute
allergic pulmonary inflammation using the transgenic mice
10   generated in example 17. This model was performed essentially
as described by McMillan *et al.* [85] and example 13. The
model was characterised at its endpoint by increased BAL and
lung tissue IL-13, cellular infiltration into the lung and
BAL, increased serum IgE levels and airways
15   hyperresponsiveness (AHR).

Model protocol
The protocol for this model was as described in Example 13
except that anti-human IL-13 antibodies were dosed instead of
20   BAK209B11.

EXAMPLE 19
Efficacy of anti-human IL-13 antibodies in the huIL-13-
transgenic Gerard murine model of acute pulmonary inflammation

25

Murine model of acute allergic pulmonary inflammation

The effect of anti human IL-13 neutralising human IgG4
antibodies were investigated in another murine model of acute
30   allergic pulmonary inflammation using the transgenic mice
generated in example 17. This model was performed essentially
as described by Humbles et al, [86] and in example 14. The
model is characterised at its endpoint by increased BAL and
lung tissue IL-13, cellular infiltration into the lung and

BAL, increased serum IgE levels and airways
hyperresponsiveness (AHR).

Model protocol
5   The protocol for this model was as described in
    Example 14 except that anti-human IL-13 antibodies were dosed
    instead of BAK209B11.

EXAMPLE 20
10 *Efficacy of anti-human IL-13 antibodies in the huIL-13-
    transgenic Lloyd chronic model of pulmonary inflammation*

    The effect of anti human IL-13 neutralising human IgG4
    antibodies were investigated in a model of chronic allergic
15 pulmonary inflammation using the transgenic mice generated in
    example 17. This model was performed essentially as described
    by Temelkovski *et al.* [87] and in Example 15 and is
    characterised at its endpoint by cellular infiltration into
    the lung and BAL, increased serum IgE levels and airways
20 hyperresponsiveness (AHR).

Model protocol   .
    The protocol for this model was as described in Example 15
    except that anti-human IL-13 antibodies were dosed instead of
25 BAK209B11

EXAMPLE 21
    *Pharmacokinetics and pharmacodynamics of anti-human IL-13
    antibodies in Ascaris.suum-allergic cynomolgus monkeys*

30
    The pharmacokinetics and pharmacodynamics of 502G9 were
    evaluated in 4 allergic but non-challenged cynomolgus primates
    (2 male/2 female) after a single 10mg/kg i.v bolus dose. The
    experiment ran for 29 days. The antibody's pharmacokinetic

parameters were determined from a geomean average serum-drug concentration curve and are detailed below in Table 4.

In the same study serum IgE concentrations were also followed
5   using a human IgE ELISA kit (Bethyl laboratories, USA).

Results
Serum IgE concentrations were significantly reduced after a single 10mg/kg i.v bolus dose of BAK502G9, from 100 % control
10   levels (predose) to 66 ± 10% of control values (p<0.05), at 4 and 5 days after dosing. This lowering of serum IgE concentration recovered to 88 ± 8 % of control levels by day 22 (see Figure 20). Again these data were derived by normalising each animals serum IgE concentration to predose
15   levels, where predose concentrations was 100%, and then averaging the curves from the 4 animals tested.

The two male monkeys had relatively low predose total serum IgE (60ng/mL and 67ng/mL). These IgE levels did not change in
20   a fashion suggesting a trend following treatment with BAK502G9 (Figure 20B). The two female monkeys had relatively high predose total serum IgE (1209ng/mL and 449ng/mL). These IgE levels were decreased following treatment with BAK502G9, maximally by 60% at 7 days, and returning to approximately
25   predose levels by 28 days post-administration (Figure 20B). These data provide indication that BAK502G9 lowers serum IgE concentrations in animals with relatively high circulating IgE of IgE.

30   *EXAMPLE 22*
Efficacy of anti-human IL-13 antibodies in cynomolgus models of dermal allergy

The effects of anti-human IL-13 neutralising human IgG4

antibodies were investigated in a primate model of acute
allergic dermal inflammation. This model was performed by
injecting human IL-13 and *A.suum* antigen intradermally into
cynomolgus monkeys. 24-96h later, dermal biopsies and serum
5 samples were taken. The model was characterised at its
endpoint by cellular infiltration into the skin.

EXAMPLE 23
Efficacy of anti-human IL-13 antibodies in cynomolgus models
10 *of pulmonary allergy*

The effect of anti human IL-13 neutralising human IgG4
antibodies were investigated in a primate model of acute
allergic pulmonary inflammation. This model was performed by
15 exposing *a.suum*-allergic cynomolgus primates to nebulised
a.suum antigen, thereby generating an allergic reaction. This
allergy was characterized at its end point by cellular
infiltration into the lung and BAL, increased serum IgE levels
and airways hyper-responsiveness.
20

Pharmacodynamics were additionally evaluated *ex vivo* using a
flow cytometric method. CD23 is the high affinity IgE
receptor and can be expressed on peripheral human blood
mononuclear cells. CD23 expression can be induced, in terms
25 of the number of cells expressing CD23 and also in how much
CD23 each cell expresses by both IL-13 and IL-4. The IL-13,
but not IL-4, mediated response can be inhibited by anti-human
IL-13 antibodies.

30 Animals were preselected for entry into this 2-phase study on
the basis of previously established AHR following nebulised
antigen (*ascaris suum* extract) challenge. In phase I airway
function was assessed during intravenous histamine challenge
on days 1 and 11. PC_{30}, the histamine dose required to

generate a 30% increase in lung resistance (R_L) above
baseline, was determined from each histamine dose-response
curve. On days 9 and 10, animals were challenged with
individually tailored doses of nebulised antigen previously
5   shown to generate a 40% increase in R_L as well as a 35%
decrease in dynamic compliance (C_{DYN}). Historically in this
model, a greater R_L has been observed following the second
challenge with a given allergen dose than the first; this is
antigen priming. The two antigen challenges caused AHR, as
10   measured by an increased area under the histamine dose-
response curve and/or a fall in PC_{30}, and BAL, as well as
eosinophilia at day 11 compared to day 1. Animals displaying
an AHR-phenotype were selected to enter phase II.

15   Phase II was run exactly as phase I except that all animals
received a 30mg/kg BAK502G9 infusion on days 1, 5 and 9. The
effects of BAK502G9 were assessed by comparing the changes
seen in phase II with changes seen in phase I for individual
animals.
20

Blood, serum, BAL and lung tissue were colleted. Serum IgE
levels were monitored by ELISA. Serum from BAK502G9 treated
cynomolgus monkeys was shown to inhibit the expression of CD23
on human peripheral blood mononuclear cells induced by IL-13
25   but not IL-4. The magnitude of this inhibition was consistent
with the serum BAK502G9 levels predicted by PK ELISA.

Results
BAK502G9 significantly inhibited AHR as measured by R_L AUC
30   ($p<0.05$) (Figure 26A; Table 7). An inhibitory effect of
BAK502G9 on AHR, as measured by PC_{30}, was observed but did not
reach statistical significance (Figure 26B; Table 7).
BAK502G9 also significantly inhibited both antigen priming
($p<0.01$) (Figure 26C; Table 7) and BAL inflammation. BAK502G9

significantly inhibited total cell (p<0.05) and eosinophil
(p<0.05) but not macrophage, lymphocyte or mast cell influx
into the BAL (Figure 26D; Table 7).

5 *EXAMPLE 24*
   Efficacy of anti-human IL-13 antibodies against the asthmatic
   phenotype that develops when human IL-13 is administered to
   the mouse lung

10 *Murine model of airways hyperresponsiveness*
    The efficacy of the anti-human IL-13 neutralising antibody
    BAK502G9, against the development of airways hyper-
    responsiveness (AHR) following administration of human IL-13
    to the mouse lung was investigated. This model was performed
15 essentially as described by Yang et al [119] with the
    exception that human IL-13 was used in place of murine IL-13.

    Model protocol
    To develop the phenotype, male BALB/c mice were exposed to two
20 doses of human IL-13 separated by a 48-hour interval. In
    brief, mice were anaesthetised with an intravenous injection
    of 100µl saffan solution (1:4 diluted in water). Mice were
    intubated with a 22-gauge catheter needle, through which human
    recombinant IL-13 (25 µg dissolved in 20µl phosphate-buffered
25 saline (PBS)) or vehicle control (PBS) was instilled. Airway
    function was assessed 24 hours after the last administration
    of IL-13 by increasing methacholine challenges and monitored
    using conscious plethysmography (Buxco). PC$_{200}$ (concentration
    of methacholine required to increase baseline penH by 200%)
30 was determined from 4 parameter unfixed curve fitting of
    methacholine dose-response curves. Antibody treatments were
    administered by intra-peritoneal injection 24 hours prior to
    the each dose of IL-13.

Results

Intratracheal installation of human IL-13 into naïve wild-type mice resulted in development of significant (p<0.05) airways hyperresponsiveness relative to control animals as determined
5   by PC_{200} methacholine concentrations. Systemically administered BAK502G9 (1mg/kg) significantly (p<0.01) inhibited the development of AHR whereas the null control antibody had no effect (Figure 23).

10

EXAMPLE 25

Neutralisation potency of BAK502G9 as a human IgG4 against human IL-13 dependent IgE release from human B cells.

15   *B cell switching assay protocol*

IL-13 has been shown to induce IgE synthesis in human B cells *in vitro* [120]. Factor dependent IgE release from human B cells was determined by ELISA. The neutralisation potency of BAK502G9 as a human IgG4 was assessed against human IL-13
20   dependent IgE release from human B cells.

Peripheral blood mononuclear cells (PBMC) were purified from human buffy coat (Blood Transfusion Service) by centrifugation over a 1.077g/L density gradient. B cells were purified from
25   PBMC with a B cell isolation kit II (Miltenyi Biotec), using reagents and methods described by the manufacturer. Assay media comprised Iscoves modified dulbeccos medium (Life Technologies) containing 10% foetal bovine serum and 20µg/mL human transferrin (Serologicals Proteins Inc). Following
30   purification, B cells were resuspended to a final concentration of 10^6/mL in assay media. 50µl of resuspended cells were added to each assay point in a 96 well assay plate. 50µl of 4µg/mL of the anti-CD40 antibody EA5 (Biosource) was added to assay wells as appropriate. Test solutions of

97

antibodies (six replicates) were diluted to the desired concentration in assay media. An irrelevant antibody not directed at IL-13 was used as a negative control. 50µl / well of the appropriate test antibody were added to the cells.

5   Recombinant bacterially derived human IL-13 (Peprotech) was subsequently added to a final concentration of 30ng/ml to give a total assay volume of 200µl/well. The concentration of IL-13 used in the assay was selected to give a maximal response. Assay plates were incubated for 14 days at 37°C under 5% CO_2.

10   IgE levels in the supernatant were determined by ELISA using reagents and protocols supplied by the manufacturer (BD Biosciences, Bethyl Laboratories). Data were analysed using Graphpad prism software.

15   *Results*
As demonstrated in Figure 24, BAK502G9 (VH SEQ ID NO: 15; VL SEQ ID NO: 16) was able to inhibit human IL-13 dependant IgE production by human B cells. BAK502G9 as human IgG4 had an IC_{50} of 1.8nM against 30ng/ml human IL-13.

20

EXAMPLE 26
Efficacy of BAK502G9 against IL-13 mediated potentiation of histamine induced Ca^{2+} signalling in primary human bronchial smooth muscle cells

25

IL-13 has been shown to directly modulate the contractility of airway smooth muscle [121, 122]. Intracellular calcium mobilization is a prerequisite for smooth muscle contraction. Recent studies have shown that IL-13's ability to alter smooth
30   muscle contractility is mediated in part through modulation of contractile agonist induced Ca^{2+} signaling [123, 124].

The efficacy of BAK502G9, an anti-human IL-13 antibody formatted as an IgG4, against IL-13 mediated alterations in

primary human bronchial smooth muscle cells (BSMC) signalling
responses to the contractile agonist, histamine, was
investigated in a Ca^{2+} signalling assay.

5   *BSMC Ca^{2+} signalling assay protocol*
Human primary BSMC, Smooth Muscle Growth Medium-2 (SmGM-2) and
Smooth Muscle Basal Medium (SmBM) were obtained from Bio
Whittaker. The BSMC were maintained in SmGM-2 according to
supplier's recommendations. BSMC were plated at 2 x 10⁴
10   cells/well in a 96-well microtitre cell culture plate and were
allowed to attach for 24 hours, then re-fed and incubated for
a further 24 hours. Prior to the Ca^{2+} signalling experiment,
the BSMC were stimulated with IL-13 (Peprotech) at 50ng/ml
final concentration with or without antibody and incubated for
15   18-24 hours. BAK502G9 and an isotype matched irrelevant
control monoclonal antibody, CAT-001, were evaluated at a
final concentration of 10µg/ml. Changes in intracellular Ca^{2+}
concentrations in response to histamine (Calbiochem), titrated
from 20µM, were measured using standard techniques with the
20   Ca^{2+} sensitive dye Fluo-4 (Molecular Probes) and a 96-well
Fluorescence Imaging Plate Reader (FLIPR) (Molecular Devices).
The area under the curve (AUC) of the Ca^{2+} signalling response
to histamine was determined for each cell pre-treatment
condition. Data analyses were performed using GraphPad Prism
25   version 4 for Windows (GraphPad Software).

Results
Pre-incubation of BSMC with IL-13 significantly enhanced Ca^{2+}
signalling in response to histamine. Pre-incubation of
30   BAK502G9 (Figure 25B)(but not an irrelevant isotype control
antibody (Figure 25A)) with IL-13 significantly inhibited the
potentiation of Ca^{2+} signalling in response to histamine
(Figure 25).

EXAMPLE 27

Neutralisation potency of anti-IL-13 antibodies in a human IL-13 dependent PBMC CD23 expression assay

5   The potency of a representative IL-13 antibody was evaluated in the human IL-13 dependent peripheral blood mononuclear cell (PBMC) CD23 expression assay. PBMC respond to both IL-13 and IL-4 by increasing cell surface expression of CD23 [120]. CD23 (FceRII) is the low-affinity receptor for IgE and is

10   expressed on a variety of inflammatory cells, including monocytes. Inhibition of human IL-13 dependent CD23 expression upregulation was determined by measuring the reduction in binding of fluorescently labelled CD23 monoclonal antibody to PBMCs by flow cytometry.

15

Assay protocol

Human blood was obtained from the Blood Transfusion Service and erythrocytes depleted by 40 minute dextran-T500 (Pharmacia) sedimentation (0.6% final concentration). The

20   leukocyte and platelet rich fraction was then separated by a 20 minute 1137g centrifugation over a discontinuous Percoll gradient of 3mL 64% and 5mL 80% (100% was 9 parts Percoll and 1 part 10x PBS). PBMCs were harvested from the top of the 64% layer, washed and resuspended in assay buffer (Invitrogen RPMI

25   1640, 10% FCS, 200IU/mL penicillin, 100µg/mL streptomycin, 2mM L-Glutamine). The assay was performed in 24 well plates with $2x10^6$ cells, ± 80pM recombinant human IL-13 (Peprotech) or 21pM recombinant human IL-4 (R&D Systems), ± BAK502G9 or irrelevant IgG4, in a final volume of 500mcL. Cells were

30   cultured for 48h at 37C before being harvested and stained with CD23-PE (BD Pharmingen) for 20 minutes at 4C. Finally, cells were read on a flow cytometer. CD23 expression was determined by CD23 'score'; percent of CD23 positive cells multiplied by the 'brightness' of the stain (geomean

fluorescence). No stimulant CD23 'score' was subtracted and
data presented as a percentage of the response to IL-13 alone
(100%). Data has been expressed as the mean ± SEM drawn from
4-6 separate experiments, using cells from 4-6 individual
5   donors, performed in triplicate for each point.

Results
Incubation of PBMC with 80pM IL-13 or 21pM IL-4 for 48 hours
resulted in clear CD23 expression (Figure 27 and Figure 28).
10   BAK502G9 dose-dependently inhibited IL-13-induced CD23
expression with a geometric mean of 120.2pM (Figure 27). In
contrast, BAK502G9 was unable to inhibit the CD23 expression
induced by 21.4pM IL-4 (n=4 from individual donors, Figure
28). Irrelevant IgG4 did not inhibit either IL-13 or IL-4
15   dependent CD23 expression on PBMC (Figure 27 and Figure 28).
Co-stimulation of PBMC with 80pM IL-13 and 21.4pM IL-4,
produced an additive CD23 response. BAK502G9, but not CAT-
001, reduced CD23 expression levels to those seen with IL-4
stimulation alone (Figure 28).
20

EXAMPLE 28
Neutralisation potency of a human IL-13 antibody in a human
IL-13 dependent eosinophil shape change assay

25   The aims of this study were to evaluate the effect of IL-13
antibodies on eosinophil shape change induced by mediators
released from NHLF following stimulation with factors found in
the lungs of asthmatics such as IL-13 [125,126], TNF-α [127],
TGF-β1 [128]. IL-13 synergises with TNF-α [129] or TGF-β1
30   [130] to induce fibroblasts to produce eotaxin-1, which can
then act to directly chemoattract eosinophils. Leukocyte
shape change responses are mediated through rearrangements of
the cellular cytoskeleton and are essential to the processes
of leukocyte migration from the microcirculation into sites of

inflammation. Inhibition of IL-13-dependent shape-change-inducing factor release by NHLFs was determined by measuring the reduction in eotaxin-1secretion by ELISA and reduction in eosinophil shape change by flow cytometry.

Assay protocol
NHLF cells were cocultured with media alone or media containing stimulants (9.6nM IL-13, 285.7pM TNF-α (R&D Systems) and 160pM TGF-β1 (R&D Systems) in the absence or presence of BAK502G9 (concentration range 875nM - 6.84nM). Cells were then cultured for a further 48h at 37°C before the resulting conditioned media was aspirated and stored at -80°C. The concentration of eotaxin-1 in conditioned media was assessed using the R&D systems Duoset ELISA system (R&D Systems).

Human blood was obtained from the Blood Transfusion Service and erythrocytes depleted by 40 minute dextran-T500 (Pharmacia) sedimentation (0.6% final concentration). The leukocyte and platelet rich fraction was then separated by a 20 minute 1137g centrifugation over a discontinuous Percoll gradient of 3mL 64% and 5mL 80% (100% was 9 parts Percoll and 1 part 10x PBS). Granulocytes were harvested from the 64%:80% interface, washed and resuspended in assay buffer (Sigma PBS, 1mM CaCl2, 1mM MgCl2, 10mM HEPES, 10mM D-glucose, 0.1% Sigma BSA, pH 7.3). The assay was performed in FACS tubes with 5x10^5 cells, ± 3nM recombinant human eotaxin-1 (R&D Systems) or conditioned media, in a final volume of 400μL. Cells were incubated for 8.5 minutes at 37C before being transferred to 4^{0}C and fixed with a fixing agent (CellFix, BD Biosciences) and finally read on a flow cytometer. Eosinophils were identified by their FL-2 autofluorescence and the forward scatter (FSC) parameter read. Eosinophil FSC changed in response to both eotaxin-1 and conditioned media providing

measurement of shape change. Tubes were sampled at high flow
rate and acquisition was terminated after 1000 eosinophil
events or 1 minute, whichever was the sooner. Shape change
was calculated as a percentage of the FSC caused by shape

5   change buffer alone (100% blank shape change). Data have been
expressed as the mean % blank shape change ± SEM drawn from 4
separate experiments. Each experiment used cells from an
individual buffy coat (and hence individual donor), performed
in duplicate for each point.

10

Results

NHLF cells co-stimulated with 9.6nM IL-13, 285.7pM TNF- α and
160pM TGF-β1 and cultured for 48h secreted 9.6nM eotaxin-1
into the culture media. In contrast, NHLF cells cultured only

15   with maintenance media secreted 0.1nM eotaxin-1 into the
culture media. This eotaxin-1 production was IL-13 dependent
as IL-13/TNF-α/TGF-β1 co-stimulated NHLF cell eotaxin-1
production was dose-dependently inhibited by BAK502G9 with an
IC_{50} of 32.4nM (Figure 29A).

20

The primary aim of this part of the study was to examine
eosinophil shape change. The magnitude of eosinophil shape
change in response to 3nM eotaxin (positive control) was
122.2±2.1% (n=4). Eotaxin-1 induced shape change was

25   completely inhibited by 100nM of an anti-eotaxin antibody CAT-
213, mean shape change 101.0±1.0% (n=4).

Media from NHLF cells co-stimulated with 9.6nM IL-13, 285.7pM
TNF-α and 160pM TGF-β1 and cultured for 48h (conditioned

30   media), induced a clear eosinophil and shape change (Figure
29B). In contrast, media from NHLF cultured for 48h in NHLF
maintenance media alone did not induce eosinophil shape change
(Figure 29B).

The addition of anti-IL-13 antibody BAK502G9 to co-stimulated media prior to NHLF culture, resulted in a dose-dependent inhibition of eosinophil shape change, with a geometric mean IC_{50} of 16.8nM when assayed at 1:16 dilution (Figure 29B).

5

The ability of stimulants (IL-13, TNF-α and TGF-β1) not cultured with NHLF cells to induce eosinophil and neutrophil shape change was also investigated. 9.6nM IL-13, 285.7pM TNF-α and 160pM TGF-β1 did not induce a clear eosinophil shape

10   change. This suggests that the eosinophil shape change ability of conditioned media develops during NHLF cell culture with the stimulants is not due to any of the stimulants alone or in combination (Figure 29B).

15   *Example 29*
Mapping of anti-IL-13 antibodies on Human IL-13

The epitope mapping of a representative IL-13 antibody BAK502G9 was performed using a molecular approach and standard

20   peptide excision.

Molecular Approach
IL-13 chimaeras were engineered, where parts of the human IL-13 sequence were replaced with murine sequence. These chimeras

25   were used in binding studies with representative IL-13 antibodies to help identify the specific epitope.

Two panels of IL-13 chimaeras were produced. The first panel contained nine chimaeras (Figure 30) and was used to locate

30   the general position of the epitope. The second panel contained ten chimaeras (Figure 31) and was used to fine map the epitope.

The chimaeric IL-13 sequences were assembled using PCR and
cloned into a Gateway® entry vector, which were then
recombined with a destination vector pDEST8 (modified to code
for a detection and affinity tag at the C-terminus of the

5   recombinant protein). These expression vectors were used to
transform DH10Bac™ chemically competent E coli allowing site-
specific transposition of tagged chimeric IL-13, into the
baculovirus shuttle vector (bacmid). Recombinant bacmid DNA
was isolated for each IL-13 chimera and transfected into Sf9

10   (Spodoptera frugiperda) insect cells using Cellfectin®
Reagent. Recombinant baculovirus was harvested 72 hours post-
transfection and passaged through Sf9 insect cells twice more.

Insect 2000-500 ml culture supernatant was purified on an
15   affinity column and eluted material was concentrated from 16
to 1 ml and loaded on a size exclusion Superdex 200 HR10/300GL
column for final polishing and buffer exchange.

A homogenous competition assay using biotinylated human IL-13,
20   streptavidin-anthophyocynate and Europium labelled BAK502G9
was developed. The assay is as follows: Eu-BAK502G9 binds
biotinylated—human IL-13, the complex is then recognised by
the streptavidin APC conjugate and when a flash of light is
applied the energy is transferred from the APC label to the
25   Europium by proximity, and time resolved florescence can be
measured. Competition for this binding is introduced by way of
the un-labelled human IL-13 (as control) and the chimeric
constructs. This competition is quantified to calculate the
relative affinities of the IL-13 mutants for IL-13 antibodies
30   enabling mutations altering binding to be identified.

Results
Chimeric construct IL13-Helix D (Table 5) was found to be the
weakest competitor against biotinylated human IL-13 for

binding BAK502G9, indicating that helixD within the IL-13
molecule was involved with BAK502G9 epitope binding (Table 5)
Reduced activity was also seen for the 4041 and 3334 mutants
where residues 40, 41, and 33, 34 of the parent sequence
5   respectively were changed indicating potential involvement of
helixA in the recognition of BAK502G9. The reduced activities
of loop3 was discounted as this loop has a reduced number of
amino acids in the mutant as compared to the human molecule
and is likely to alter the overall structure of the protein.
10   Other reductions in the ability of the chimeric IL-13
molecules to compete for BAK502G9 binding were not considered
significant for such amino acid changes.

A more targeted set of mutations within helix D (Figure 26)
15   were then tested. Results obtained are demonstrated in Table 6
and are as follows:

Results show that chimeric constructs 116117TK (where lysine
at position 116 was replaced with threonine and the aspartate
20   at position 117 was replaced with lysine), 123KA (where lysine
at position 123 was replaced) and 127RA (where arginine at
position 127 was replaced) are least able to compete for
binding to BAK502G9 (123KA and 127RA do not compete at 1 µM).
Other residues implicated in binding to BAK502G9 due to their
25   reduced effectiveness in the competition assay include the
helixD residues 124Q (here lysine has been replaced with
glutamine) and 120121SY (a leucine histidine pair has been
changed to a serine tyrosine pair). Mutation of leucine at
position 58L also reduces binding and analysis of the 3D
30   structures revealed that this residue packs against helixD and
may either be directly contacted by BAK502G9 or may affect the
alignment of helixD.

These experiments demonstrate that residues within helixD are critical for the binding of BAK502G9 to IL-13. In particular the lysine at position 123 and the arginine at position 127 are critical for this binding as mutation to either abolishes
5   binding of BAK502G9.

Epitope Excision
The epitope mapping of BAK502G9 was also performed using the standard peptide excision procedure. Here IgG is immobilised
10   onto solid phase and allowed to capture the IL-13 ligand. The formed complex is then subject to specific proteolytic digestion, during which accessible peptide bonds are cleaved, however those protected by the IgG: ligand interface remain intact. Thus, a peptide containing the epitope remains bound
15   to the IgG. This can then be desorbed, collected and identified by mass spectrometry (ms).

Two complementary techniques were used, the first made use of the Ciphergen ProteinChip Reader MALDI-TOF mass spectrometer,
20   where it was possible to covalently link the IgG to a mass spectrometer chip and then perform the digestion and extraction *in-situ*. The second technique used biotinylated BAK502G9 linked to streptavidin coated beads and allowed the collection of sufficient peptide for sequence confirmation by
25   tandem mass spectrometry (ms/ms).

The two procedures although differing in absolute detail and scale involved essentially the same steps, coupling of the IgG, blocking of unreacted binding sites, washing, ligand
30   capture, removal of unbound ligand, digestion and a final washing step.

The MALDI-TOF ms approach made use of proprietary ms chips activated with carbonyldiimidazole that covalently binds to

free primary amine groups to which the IgG at 1-2 mg/ml in PBS
was coupled to overnight at 4°C. The chip was subsequently
blocked with an ethanolamine solution at room temperature for
1 hour and then washed extensively with PBS or HBS plus a

5   suitable detergent. A one picomole aliquot of IL-13 was then
applied to the chip in either PBS or HBS and allowed to bind
to the chemically immobilized IgG for 2 hours at room
temperature. This was followed by further washes in PBS or HBS
with and without detergent to remove any non-specifically

10   bound IL-13. A solution of trypsin ranging from 200 to
3.1µg/ml in PBS or HBS was then applied to the IgG:ligand
complex and digestion allowed to proceed for 30 minutes at
room temperature after which the chip was washed in PBS or HBS
plus detergent, PBS or HBS and finally water. After

15   application of a suitable MALDI-TOF ms matrix the chip was
then be placed directly in the mass spectrometer and analysed.

The bead based approach started with the biotinylation of the
IgG, using an NHS biotin compound, at a molar ratio of 1 IgG

20   to 4 biotin molecules. Removal of unbound biotin and the by-
products of the reaction using gel filtration followed this.
The biotinylated IgG was then allowed to bind to neutravidin
coated agarose beads, where it was attempted to maximize the
IgG capture. Aliquots of IgG coated beads were then dispensed

25   into a concentrator spin columns and washed with Dulbecco's
PBS + 0.05% Tween 20 followed by resuspension in Dulbecco's
PBS + 0.05% Tween 20. A pulse of IL-13 was then applied to the
resuspended IgG beads and binding allowed to proceed for 10
minutes after which the liquid phase was removed by

30   centrifugation and the beads washed with Dulbecco's PBS +
0.05% Tween 20 followed by resuspension in Dulbecco's PBS +
0.05% Tween 20.

The bead:IgG:ligand complex was then subject to proteolysis
with either trypsin or chymotrypsin with incubation at room
temperature or 37°C. After which the beads were again washed
in Dulbecco's PBS + 0.05% Tween 20 followed by a further
5   washes in Dulbecco's PBS without detergent. The beads were
then resuspended in a water, acetonitrile, trifluroacetic mix
and the supernatant recovered. This was then variously
analysed either by MALDI-TOF ms or by reverse phase HPLC mass
spectrometry, including tandem (ms/ms) fragmentation using the
10   ThermoQuest LCQ ESI ion-trap mass spectrometer. An attempt
was then made to match the resulting fragmentation pattern to
the human IL-13 sequence and the separate heavy and light
chain sequence of BAK502G9 IgG.

15   During the experimental sequence a number of controls,
primarily blank surfaces, IgG only and isotype controls were
employed to demonstrate that the identified peptides were
derived specifically from IgG captured IL-13 and not a product
of BAK502G9 or non-specifically bound IL-13 digestion.
20

Results
The experimental series consistently gave single IL-13
specific peptides for each digestion. Data from the LCQ ion
trap instrument revealed that the tryptic fragment had a
25   monoisotopic mass of 3258Da (MH+) and the chymotrypsin
fragment a monoisotopic mass of 3937Da (MH+).

A search of these masses against the appropriate in silico
digestion of human IL-13 gave close matches to related
30   peptides in the C-terminal portion of the molecule.

Match for trypsin peptide mass: 3258Da

5 At a tolerance of 1000ppm, 3258Da matches to the sequence from
   aspartic acid at position 106 to the C-terminal asparagine at
   position 132. There are no other matches at this tolerance.
   This region is highlighted in bold on the sequence of the
   precursor form of human IL-13 below.

10 MALLLTTVIALTCLGGFASPGPVPPSTALRELIEELVNITQNQKAPLCNGSMVWSINLTAGM
   YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVR**DTKIEVAQFVKDLLLHLKK**
   LFREGRFN

    Match for chymotrypsin peptide mass: 3937Da

15
   At a tolerance of 1000ppm, 3937Da matches to the sequence from
   serine at position 99 to the C-terminal asparagine at position
   132. This region is highlighted in bold on the sequence of the
   precursor form of human IL-13 below.

20
   MALLLTTVIALTCLGGFASPGPVPPSTALRELIEELVNITQNQKAPLCNGSMVWSINLTAGM
   YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQ**FSSLHVRDTKIEVAQFVKDLLLHLKK**
   LFREGRFN

25 Both these matches show that the BAK502G9 IgG retains the C-
   terminal portion of the IL-13 molecule during proteolysis of
   the antibody:ligand complex.

   The identity of both peptides was successfully confirmed by
30 the ms/ms, neither of which showed any significant sequence
   parallels with BAK502G9. The ms/ms fragment map tailored to
   identify either Y or B ions matched 26 of 104 possible ions in
   one charge state for the trypsin peptide and 19 of 128
   possible ions for the chymotrypsin peptide. A review of all

charge states shows identification of 23 of the 27 amino acid residues for the trypsin fragment and 29 of the 33 residues for the chymotrypsin fragment. This is sufficient to confirm identity.

The experimental sequence as a whole has identified that part of the BAK502G9 epitope on human IL-13 as lying within the twenty-seven C-terminal amino acid residues. These findings corroborate the finding of the molecular approach detailed above.

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Table 1

Kabat numbering	HCDR1				HCDR2															HCDR3										Linker	LCDR1												LCDR2							LCDR3								Mean TFI (nM)	TFI sign
BAK0278D8	PD																																																							44	
BAK0502G9	PD																																																							0.1	2
BAK1187B4	PD																																																							0.2	2

Table 1 (cont'd)

Kabat numbering		HCDR1	HCDR2	HCDR3	Linker	LCDR1	LCDR2	LCDR3	Mean TF1 (pM) app	TF1 app
BAK278D6									44	
BAK502G9									6	
BAK1063F1	RD								20.0	1
BAK049B88	PD								21.0	4
BAK101DC5	RD								22.0	1
BAK049B14	PD								22.0	3
BAK050B1B	PD								23.0	2
BAK104907	RD								23.0	1
BAK053E2	PD								23.1	3
BAK043C4	PD								23.3	4
BAK100B9	RD								27.0	1
BAK049C8	PD								28.2	5
BAK044E8	PD								28.3	3
BAK047E3	RD								30.0	1
BAK047207	PD								31.0	1
BAK100B47	RD								32.0	1
BAK103B4	RD								34.0	1
BAK049A4	PD								34.6	5
BAK078205	PD								38.3	3
VK050203	PD								41.5	2
VK100TH9	RD								45.0	1
VAK100A3	RD								45.0	1
BAK104901	D								46.0	1
BAK048482	RD								28.6	5
BAK050205	PD								29.3	4

Phage Display	PD
Ribosome Display	RD
Point mutations	O
Germlining	GL

Table 2

Binding specificity of anti-human IL-13 antibodies

	Human IL-13	Human IL-13 variant	Non-human primate IL-13
BAK278D6	+	+	+
BAK502G9	+	+	+
BAK615E3	+	-	-

5

Table 3a

Kinetic analysis of anti-human IL-13 antibodies

IgG	Off-rate (s^{-1})	On-rate $(M^{-1} s^{-1})$	KD (pM)
BAK278D6	$7.41e^{-3}$	$5.49e^{5}$	13500
BAK502G9	$4.09e^{-4}$	$2.49e^{6}$	178
BAK1167F2	$4.05e^{-4}$	$2.99e^{6}$	136
BAK1183H4	$3.00e^{-4}$	$3.7e^{6}$	81

10

Table 3b

Kinetic analysis of anti-murine IL-13 antibodies

IgG	Off-rate (s^{-1})	On-rate $(M^{-1} s^{-1})$	KD (pM)
BAK209B11	$1.98e^{-2}$	$3.9e^{6}$	5100

15

Table 4

Pharmacokinetics of BAK502G9 in 4 allergic but non-challenged
cynomolgus primates (2 male/2 female) after a single 10mg/kg
5   *i.v bolus dose over 29 days. BAK502G9 levels in serum were*
measured by ELISA (mean data).

C_{max} (t=0.25h) (μg/mL)	349.04	
Vd_{inf} (mL.kg^{-1})	75.03	<80mL/kg, infers no tissue binding.
Cl_{inf} (mL.hr^{-1}.kg^{-1})	0.23	
AUC_{inf} (mg.h.mL^{-1})	42.99	
AUC_{ext} (%)	17.34	<30% so clearance and vol. of distribution should be accurate.
$T_{0.5}$ (h)	223.55	

$\underline{Vd}_{inf}$ = volume of distribution over time 0 - infinity,
10   calculated from the extrapolated AUC.

$\underline{Cl}_{inf}$= clearance over time 0 - infinity, calculated from the
extrapolated AUC.

$\underline{AUC}_{inf}$ = area under the curve (measure of total drug exposure)
over time 0 - infinity, including an extrapolated term based
15   on the elimination rate constant (k) and the last observed
serum drug concentration.

$\underline{AUC}_{ext}$ = percentage of the total AUC that is extrapolated.

$\underline{T}_{0.5}$ = Drug half-life in the terminal elimination phase.

20

Table 5

First set of Chimeric constructs

Chimeric constructs	IC50 nM
BAK502G9	0.17 ± 0.07
loop1	0.71 ± 0.35
hum-flag	1.30 ± 0.18
30R	1.76 ± 0.45
3738VN	1.89 ± 1.9
helixB	2.49 ± 0.88
helixC	4.11 ± 0.70
loop3	5.45 ± 3.96
4041	12.02 ± 1.3
3334	12.17 ± 1.2
helixD	110.07 ± 9.9

5

Table 6

Second Set of Chimeric Constructs

Chimeric Constructs	IC50 nM
BAK502G9	0.11± 0.04
113H	1.6 ± 0.5
128H	1.6± 1.0
119LA	1.96 ± 1.0
130P	2.22 ± 0.8
120121SY	4.73 ± 1.5
58LA	5.2 ± 2.0
124Q	18.7 ± 15.9
116117T	82 ±11.3
123KA	none
127RA	none

Table 7:

Effects of BAK502G9 on various predefined endpoints.

Parameter	Phase I change	N	Phase II change	N	Endpoint
AHR (R_L AUC)	0.020±0.003	14[a]	0.004±0.006	14[a]	-0.016±0.006*
AHR (PC_{30})	-1.343±0.318	18[b]	-1.061±0.244	18[b]	0.282±0.179
Antigen priming (R_L AUC)	0.159±0.033	20[c]	0.033±0.025	20[c]	-0.126±0.043**
BAL total cells	20.623±3.160	21[d]	14.597±1.951	21[d]	-6.026±2.194*
BAL eosinophils	18.453±3.009	21[d]	13.412±1.737	21[d]	-5.041±2.090*
BAL mononuclear cells	2.050±0.438	21[d]	1.176±0.481	21[d]	-0.874±0.506

21 animals displaying AHR (PC_{30}) in Phase I and an additional
animal with an antigen priming phenotype were carried forward
for testing in Phase II (22 in total). Not every animal had
AHR as measured by both AUC and PC_{30}. Only animals which
displayed AHR in phase I and whose AHR was assessed in both
Phase I and Phase II were included in the AHR results.
Statistical testing was performed using InStat. Testing was a
2-way student's t-test against the null hypothesis that the
endpoint did include the number 0 (i.e. there was no change in
phase II compared to phase I); *p<0.05, **p<0.01. Data are
shown as arithmetic mean ± SEM (n=14-21).
[a]5 animals were excluded from the AUC analysis as they did not
display AHR (increased AUC) in Phase I.
3 further animals were excluded due to a technical failure in
Phase II airway function data collection.
[b]3 animals were excluded from PC_{30} analysis due to a technical
failure in Phase II airway function data collection (same
animals as in a). The additional animal with antigen priming
phenotype was excluded as it did not display PC_{30} AHR in Phase
I.

[c]2 animals were excluded from the antigen priming analysis as there was a technical failure in Phase I airway function data collection.

[d]1 animal was excluded from the BAL analysis due to marked BAL

5   inflammation at study initiation.

BAK278D6
HEAVY CHAIN

CDR1- SEQ ID NO 1:   NYGLS
CDR2- SEQ ID NO 2:   WISANGDTNYGQEFQG
CDR3- SEQ ID NO 3:   DSSSNWARWFFDL

BAK278D6
LIGHT CHAIN

CDR1- SEQ ID NO 4:   GGNNIGSKLVH
CDR2- SEQ ID NO 5:   DDGDRPS
CDR3- SEQ ID NO 6:   QVWDTGSDPVV

BAK502G9
HEAVY CHAIN

CDR1-SEQ ID NO 7:   NYGLS
CDR2-SEQ ID NO 8:   WISANGDTNYGQEFQG
CDR3-SEQ ID NO 9:   DSSSSWARWFFDL

LIGHT CHAIN

CDR1-SEQ ID NO 10:   GGNIIGSKLVH
CDR2-SEQ ID NO 11:   DDGDRPS
CDR3-SEQ ID NO 12:   QVWDTGSDPVV

BAK278D6
HEAVY CHAIN DOMAIN

SEQ ID NO 13:
EVQLVQSGAEVKKPGASVKVSCKASGYTFRNYGLSWVRQAPGQGLEWMGWISANNGDTN
YGQEFQGRITMTTETSTNTAHMELRSLRSDDTAVYYCVRDSSSNWARWFFDLWGKGTMV
TVSS

BAK278D6
LIGHT CHAIN DOMAIN

SEQ ID NO 14:
SYVLTQPPSVSVAPGQTARIPCGGNNIGSKLVHWYQQKPGQAPVLVVYDDGDRPSGIPE
RFSGSNSGNTATLTISRIDAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK502G9
HEAVY CHAIN DOMAIN

SEQ ID NO 15:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISANNGDTN
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK502G9
LIGHT CHAIN DOMAIN

SEQ ID NO 16:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK278D6
HEAVY CHAIN

FR1- SEQ ID NO 17:   EVQLVQSGAEVKKPGASVKVSCKASGYTFR
FR2- SEQ ID NO 18:   WVRQAPGQGLEWMG
FR3- SEQ ID NO 19:   RITMTTETSTNTAHMELRSLRSDDTAVYYCVR

BAK278D6
LIGHT CHAIN

FR1- SEQ ID NO 20:   SYVLTQPPSVSVAPGQTARIPC
FR2- SEQ ID NO 21:   WYQQKPGQAPVLVVY
FR3- SEQ ID NO 22:   GIPERFSGSNSGNTATLTISRIDAGDEADYYC

BAK167A11
HEAVY CHAIN DOMAIN

SEQ ID NO 23:
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTY
YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVGAAGEGYYGYWGRGTLVTV
SS

BAK167A11
LIGHT CHAIN DOMAIN

SEQ ID NO 24:
NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYDDNQRPSGV
PDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSNNDVFGGGTKVTVL

BAK209B11
HEAVY CHAIN DOMAIN

SEQ ID NO 25:
QVQLQESGGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVSSISASGDST
FYADSVKGRFTISRDNNKNMVFLQVNSLRADDTAVYFCAKDWSQWLVGDAFDVWGRGTT
VTVSS

BAK209B11
LIGHT CHAIN DOMAIN

SEQ ID NO 26:
DIQLTQSPSTLSASVGDRVTITCRASQSVSLWVAWYQQRPGKAPKLLIYDGSTLQSGVP
ARFSGSGSGTEFTLTISSLQPDDFATYYCQQYKTFSTFGQGTKVEIKRA

BAK502G9
HEAVY CHAIN

FR1- SEQ ID NO 27:   QVQLVQSGAEVKKPGASVKVSCKASGYTFT
FR2- SEQ ID NO 28:   WYRQAPGQGLEWMG
FR3- SEQ ID NO 29:   RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR

BAK502G9
LIGHT CHAIN

FR1- SEQ ID NO 30:   SYVLTQPPSVSVAPGKTARITC
FR2- SEQ ID NO 31:   WYQQKPGQAPVLVIY
FR3- SEQ ID NO 32:   GIPERFSGSNSGNTATLTISRVEAGDEADYYC

BAK615E3
HEAVY CHAIN DOMAIN

SEQ ID NO 33:
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTY
YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVGKATTEEGYYGYWGRGTLV
TVSS

BAK615E3
LIGHT CHAIN DOMAIN

SEQ ID NO 34:
NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYDDNQRPSGV
PDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSNNDVFGGGTKVTVL

BAK1167F2

HEAVY CHAIN DOMAIN

SEQ ID NO 35:
QVQLVQSGAEVKKPGASVKVSCKASGYTFEQTGVSWVRQAPGQGLEWMGWISANNGDTN
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1167F2
LIGHT CHAIN DOMAIN

SEQ ID NO 36:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1183H4
HEAVY CHAIN DOMAIN

SEQ ID NO 37:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWINYDGGNTQ
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1183H4
LIGHT CHAIN DOMAIN

SEQ ID NO 38:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1105H3
HEAVY CHAIN DOMAIN

SEQ ID NO 39:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISGLNGETL
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1105H3
LIGHT CHAIN DOMAIN

SEQ ID NO 40:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1111D10
HEAVY CHAIN DOMAIN

SEQ ID NO 41:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWIATPDGQTS
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSNSSWARWFFDLWGRGTLV
TVSS

BAK1111D10

LIGHT CHAIN DOMAIN

SEQ ID NO 42:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1167F4
HEAVY CHAIN DOMAIN

SEQ ID NO 43:
QVQLVQSGAEVKKPGASVKVSCKASGYTFIDTGVSWVRQAPGQGLEWMGWISANNGDTN
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1167F4
LIGHT CHAIN DOMAIN

SEQ ID NO 44:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1184C8
HEAVY CHAIN DOMAIN

SEQ ID NO 45:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISGSNGYTS
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1184C8
LIGHT CHAIN DOMAIN

SEQ ID NO 46:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1185E1
HEAVY CHAIN DOMAIN

SEQ ID NO 47:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWINDATGDTQ
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1185E1
LIGHT CHAIN DOMAIN

SEQ ID NO 48:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1185F8
HEAVY CHAIN DOMAIN

SEQ ID NO 49:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYGLSWVRQAPGQGLEWMGWIRNIDGYTI
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1185F8
LIGHT CHAIN DOMAIN

SEQ ID NO 50:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1187B4
HEAVY CHAIN DOMAIN

SEQ ID NO 51:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWIDDDSGTTI
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1187B4
LIGHT CHAIN DOMAIN

SEQ ID NO 52:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK1166G2
HEAVY CHAIN DOMAIN

SEQ ID NO 53:
QVQLVQSGAEVKKPGASVKVSCKASGYTFANTGISWVRQAPGQGLEWMGWISANNGDTN
YGQEFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDSSSSWARWFFDLWGRGTLV
TVSS

BAK1166G2
LIGHT CHAIN DOMAIN

SEQ ID NO 54:
SYVLTQPPSVSVAPGKTARITCGGNIIGSKLVHWYQQKPGQAPVLVIYDDGDRPSGIPE
RFSGSNSGNTATLTISRVEAGDEADYYCQVWDTGSDPVVFGGGTKLTVL

BAK167A11
HEAVY CHAIN

CDR1- SEQ ID NO 55: SYAMS
CDR2- SEQ ID NO 56: AISGSGGSTYYADSVKG
CDR3- SEQ ID NO 57: VGAAGEGYYGY

BAK167A11
LIGHT CHAIN

CDR1- SEQ ID NO 58: TRSSGSIASNYVQ
CDR2- SEQ ID NO 59: DDNQRPS
CDR3- SEQ ID NO 60: QSYDSNNDV

BAK1167F2
HEAVY CHAIN

CDR1- SEQ ID NO 61: QTGVS
CDR2- SEQ ID NO 62: WISANGDTNYGQEFQG

CDR3- SEQ ID NO 63: DSSSSWARWFFDL

BAK1167F2
LIGHT CHAIN

CDR1- SEQ ID NO 64: GGNIIGSKLVH
CDR2- SEQ ID NO 65: DDGDRPS
CDR3- SEQ ID NO 66: QVWDTGSDPVV

BAK1166G2
HEAVY CHAIN

CDR1- SEQ ID NO 67: NTGIS
CDR2- SEQ ID NO 68: WISANGDTNYGQEFQG
CDR3- SEQ ID NO 69: DSSSSWARWFFDL

BAK1166G2
LIGHT CHAIN

CDR1- SEQ ID NO 70: GGNIIGSKLVH
CDR2- SEQ ID NO 71: DDGDRPS
CDR3- SEQ ID NO 72: QVWDTGSDPVV

BAK1184C8
HEAVY CHAIN

CDR1- SEQ ID NO 73: NYGLS
CDR2- SEQ ID NO 74: WISGNGYTSYGKEFQG
CDR3- SEQ ID NO 75: DSSSSWARWFFDL

BAK1184C8
LIGHT CHAIN

CDR1- SEQ ID NO 76: GGNIIGSKLVH
CDR2- SEQ ID NO 77: DDGDRPS
CDR3- SEQ ID NO 78: QVWDTGSDPVV

BAK1185E1
HEAVY CHAIN

CDR1- SEQ ID NO 79: NYGLS
CDR2- SEQ ID NO 80: WINDTGDTQYGQEFQG
CDR3- SEQ ID NO 81: DSSSSWARWFFDL

BAK1185E1
LIGHT CHAIN

CDR1- SEQ ID NO 82: GGNIIGSKLVH
CDR2- SEQ ID NO 83: DDGDRPS
CDR3- SEQ ID NO 84: QVWDTGSDPVV

BAK1167F4
HEAVY CHAIN

CDR1- SEQ ID NO 85: DTGVS
CDR2- SEQ ID NO 86: WISANGDTNYGQEFQG
CDR3- SEQ ID NO 87: DSSSSWARWFFDL

BAK1167F4
LIGHT CHAIN

CDR1- SEQ ID NO 88: GGNIIGSKLVH
CDR2- SEQ ID NO 89: DDGDRPS
CDR3- SEQ ID NO 90: QVWDTGSDPVV

BAK1111D10
HEAVY CHAIN

CDR1- SEQ ID NO 91: NYGLS
CDR2- SEQ ID NO 92: WIATDGQTSYGQEFQG
CDR3- SEQ ID NO 93: DSSSSWARWFFDL

BAK1111D10
LIGHT CHAIN

CDR1- SEQ ID NO 94: GGNIIGSKLVH
CDR2- SEQ ID NO 95: DDGDRPS
CDR3- SEQ ID NO 96: QVWDTGSDPVV

BAK1183H4
HEAVY CHAIN

CDR1- SEQ ID NO 97: NYGLS
CDR2- SEQ ID NO 98: WINYGGNTQYGQEFQG
CDR3- SEQ ID NO 99: DSSSSWARWFFDL

BAK1183H4
LIGHT CHAIN

CDR1- SEQ ID NO 100: GGNIIGSKLVH
CDR2- SEQ ID NO 101: DDGDRPS

CDR3- SEQ ID NO 102: QVWDTGSDPVV

BAK1185H8
HEAVY CHAIN

CDR1- SEQ ID NO 103: DYGLS
CDR2- SEQ ID NO 104: WRINDGYTIYGQEFQG
CDR3- SEQ ID NO 105: DSSSSWARWFFDL

BAK1185H8
LIGHT CHAIN

CDR1- SEQ ID NO 106: GGNIIGSKLVH
CDR2- SEQ ID NO 107: DDGDRPS
CDR3- SEQ ID NO 108: QVWDTGSDPVV

BAK278D6
HEAVY CHAIN- SEQ ID NO: 109

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAAT
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAACTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK278D6
LIGHT CHAIN- SEQ ID NO:110

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGTAAGACGGCCAGGAT
TACCTGTGGGGGAAACAACATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK502G9
HEAVY CHAIN- SEQ ID NO:111

CAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAAT
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG

ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK502G9
LIGHT CHAIN- SEQ ID NO:112

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK1105H03
HEAVY CHAIN- SEQ ID NO: 113

CAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCTCCGGCTTGAACGGCGAGACATTG
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK1105H03
LIGHT CHAIN- SEQ ID NO: 114

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK1111D10
HEAVY CHAIN- SEQ ID NO:115

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCGCAACCCCAGACGGCCAGACAAGC
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAACAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK1111D10
LIGHT CHAIN- SEQ ID NO:116

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK 1167F2
HEAVY CHAIN- SEQ ID NO: 117

CAAGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTGAGCAGACCGGCGTCTCCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAAT
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK 1167F2
LIGHT CHAIN- SEQ ID NO:118

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK 1167F04
HEAVY CHAIN- SEQ ID NO:119

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTATCGACACCGGGGTCTCCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAAT
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK 1167F04
LIGHT CHAIN- SEQ ID NO:120

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG

GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK 1183H4
HEAVY CHAIN- SEQ ID NO:121

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACTACGACGGCGGCAACACACAG
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK 1183H4
LIGHT CHAIN- SEQ ID NO:122

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK1184C8
HEAVY CHAIN- SEQ ID NO:123

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGGGAGCAACGGCTACACATCT
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACGTCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK1184C8
LIGHT CHAIN- SEQ ID NO:124

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC

CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK1185E1
HEAVY CHAIN- SEQ ID NO:125

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACGACGCCACCGGCGACACACAG
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK1185E1
LIGHT CHAIN- SEQ ID NO:126

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK1185F8
HEAVY CHAIN- SEQ ID NO:127

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAGATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTAGAGTGGATGGGATGGATCCGCAACATCGACGGCTACACAATT
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK1185F8
LIGHT CHAIN- SEQ ID NO:128

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK1187B4
HEAVY CHAIN- SEQ ID. NO:129

CAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTACAAATTATGGTCTCAGCTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCGACGACGACAGCGGCACGACAATA
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGCCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK1187B4
LIGHT CHAIN- SEQ ID NO:130

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK1166G02
HEAVY CHAIN- SEQ ID NO: 131

CAAGTGCAGTTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGTTACACCTTTGCGAACACCGGGATCTCGTGGGTGCGACAGG
CCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTAATAATGGCGACACAAAT
TATGGACAGGAATTCCAGGGCAGAGTCACCATGACCACAGATACATCCACGAGCACAGC
CTACATGGAGTTGAGGAGCCTGAGATCTGACGACACGGCCGTTTATTACTGTGCGAGAG
ACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTCTGGGGTCGGGGGACACTGGTC
ACCGTCTCCTCA

BAK1166G02
LIGHT CHAIN- SEQ ID NO:132

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGAT
TACCTGTGGGGGAAACATCATTGGAAGTAAACTTGTACACTGGTACCAGCAGAAGCCAG
GCCAGGCCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAG
CGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGC
CGGGGATGAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTAT
TCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

BAK165E7
HEAVY CHAIN- SEQ ID NO: 133

141

EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGLSWVRQAPGQGLEWMGWISANNGETN
YGQEFQGRVTMTTETPTNTAHMELRSLTSDDTAVYYCVRDSSSNWARWYFDLWGQGTLV
TVSS

BAK165E7
LIGHT CHAIN- SEQ ID NO: 134

SYVLTQPPSVSVAPGQTARIPCGGNNIGSKLVHWYQQKPGQAPVLVVYDDGDRPSGIPE
RFSGSNSGNTATLTISRIDAGDEADYYCQVWDTGSDPVVFGGGTKLTVLG

BAK165E7
HEAVY CHAIN

CDR1- SEQ ID NO:135 NYGLS
CDR2- SEQ ID NO:136 WISANNGETNYGQEFQG
CDR3- SEQ ID NO:137 DSSSNWARWYFDL

BAK165E7
LIGHT CHAIN

CDR1- SEQ ID NO:138 GGNNIGSKLVH
CDR2- SEQ ID NO:139 DDGDRPS
CDR3- SEQ ID NO:140 QVWDTGSDPVV

BAK582F7
HEAVY CHAIN

CDR1- SEQ ID NO 141: SYAMS
CDR2- SEQ ID NO 142: AISGSGGSTYYADSVKG
CDR3- SEQ ID NO 143: VGAAGEGYYGY

BAK582F7
LIGHT CHAIN

CDR1-SEQ ID NO 144: TRSSGSIASNYVE
CDR2-SEQ ID NO 145: DDNQRPS
CDR3-SEQ ID NO 146: QSYDSNNDV

BAK612B5
HEAVY CHAIN

CDR1- SEQ ID NO 147: SYAMS
CDR2- SEQ ID NO 148: AISGSGGSTYYADSVKG

CDR3- SEQ ID NO 149: VGRATTDEGYYGY

BAK612B5
LIGHT CHAIN

CDR1- SEQ ID NO 150: TRSSGSIASNYVQ
CDR2- SEQ ID NO 151: DDNQRPS
CDR3- SEQ ID NO 152: QSYDSNNDV

BAK615E3
HEAVY CHAIN

CDR1- SEQ ID NO 153: SYAMS
CDR2- SEQ ID NO 154: AISGSGGSTYYADSVKG
CDR3- SEQ ID NO 155: VGKATTEEGYY

BAK615E3
LIGHT CHAIN

CDR1- SEQ ID NO 156: TRSSGSIASNYVQ
CDR2- SEQ ID NO 157: DDNQRPS
CDR3- SEQ ID NO 158: QSYDSNNDV

BAK0278D6
HEAVY CHAIN

CDR1- SEQ ID NO 159: AATTATGGTCTCAGC
CDR2- SEQ ID NO 160: TGGATCAGCGCTAATAATGGCGACACAAATTAT
           GGACAGGAATTCCAGGGC
CDR3- SEQ ID NO 161: GACTCCAGCAGCAACTGGGCCCGCTGGTTTTTC
           GATCTC
BAK278D6
LIGHT CHAIN

CDR1- SEQ ID NO 162: GGGGGAAACAACATTGGAAGTAAACTTGTACAC

CDR2- SEQ ID NO 163: GATGATGGCGACCGGCCCTCA
CDR3- SEQ ID NO 164: CAGGTGTGGGATACTGGTAGTGATCCCGTGGTA

BAK502G9
HEAVY CHAIN

CDR1- SEQ ID NO 165: AATTATGGTCTCAGC

CDR2- SEQ ID NO 166: TGGATCAGCGCTAATAATGGCGACACAAATTATGGACA
                     GGAATTCCAGGGC
CDR3- SEQ ID NO 167: GACTCCAGCAGCAGCTGGGCCCGCTGGTTTTTCGATCTC

<u>BAK502G9</u>
LIGHT CHAIN

CDR1- SEQ ID NO 168: GGGGGAAACATCATTGGAAGTAAACTTGTACAC
CDR2- SEQ ID NO 169: GATGATGGCGACCGGCCCTCA
CDR3- SEQ ID NO 170: CAGGTGTGGGATACTGGTAGTGATCCCGTGGTA

CH Domains- SEQ ID NO: 171

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

CL Domain- SEQ ID NO: 172
QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSK
QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS